THE SPECIFIC DYNAMIC ACTION OF GLYCINE INTRAVENOUSLY ADMINISTERED TO NEPHRECTOMIZED DOGS

A. G. EATON, SHIRLEY C. CORDILL AND J. L. GOUAUX

Department of Physiology, Louisiana State University Medical Cent
New Orleans

(Received for publication April 25, 1936)

In a previous publication (Eaton, Cordill and Gouaux, '35), we have shown that the work of the kidney, due to the excretion of urea, is not a factor in the specific dynamic action of proteins. In view of the calculations of Borsook and Winegarden ('31 a, '31 b, '31 c) we have performed further experiments to determine whether or not the kidney is responsible for any measurable part of the specific dynamic action of glycine. There is no deamination of glycine taking place in hepatectomized dogs and no specific dynamic action, as shown by the experiments of Wilhelmj, Bollman and Mann ('28). Hence, if the kidney should be responsible for any of the increased heat production, it would have to be by virtue of its action after deamination.

So far as we are aware, only two papers dealing with this subject have appeared. Strieck ('28) studied the increase in heat production after a meal of meat both before and after nephrectomy. Most of his experiments were done so long after the operation that one might raise the objection that the animals were very uremic. The amount of meat fed was usually different from that given before nephrectomy. Dock ('31) investigated the oxygen consumption of rats fed on high and low protein diets, both before and after nephrectomy. Although rats of both groups showed a decreased

heat production after nephrectomy, there was no significant difference between the two groups.

Our experiments were planned so that the specific dynamic action of intravenously injected glycine could be studied as soon after nephrectomy as possible, a level of basal metabolism having been established after the operation.

METHODS

Female dogs fasted for 24 hours previous to the experiment were used. They ranged in weight from 5.2 to 13.2 kg. In most cases, one blood sample was taken before administration of sodium amytal, which was given intraperitoneally, 60 mg. per kilogram of body weight. Additional doses of sodium amytal (50 to 100 mg. per dog) were sometimes given during the day in order to preserve a uniform degree of anesthesia. Two more blood samples were taken at approximately 1½ and 3 hours, respectively, after the administration of the sodium amytal.

As soon as the animals were well asleep, a catheter was inserted and the bladder thoroughly washed. A urine sample was collected for approximately 2 to 3 hours. A tracheal cannula was inserted and connected to a Tissot spirometer for collection of expired air. Three determinations of the basal metabolism were then made.

Six dogs were used to investigate the specific dynamic action of glycine under sodium amytal anesthesia. Ten grams of glycine were dissolved in 100 cc. of 0.9% sodium chloride and injected at a constant rate into the saphenous vein during a period of approximately 20 minutes. The solution was maintained at body temperature and the last portion washed in by means of 15 cc. of 0.9% sodium chloride.

Venous blood samples were taken hourly thereafter, and the urine for the period collected for analysis. Hourly basal metabolic determinations were made as nearly consecutively as possible, during the next 5 hours.

In the nephrectomized animals, both kidneys were removed through an anterior midline incision with as little trauma as possible. The time required varied from 4 to 15 minutes.

Three further determinations of basal metabolism were made within the next 2 hours and at this point another blood sample was taken. Then, 7.88 gm. of glycine were given as described above. It had been found by the Sorensen titration method that the average excretion of glycine in the urine of the six dogs, which were not nephrectomized, amounted to 2.12 gm. Therefore, this amount was deducted.

Three nephrectomized dogs were used as controls and given 115 cc. of 0.9% sodium chloride in the same way as the glycine solution had been administered.

Expired air was analyzed by means of an accurate Haldane apparatus and the results carefully checked. Heat production was calculated on the basis of the total R.Q. since no accurate means of determining the protein metabolism of the nephrectomized dogs is possible. Blood and urine urea nitrogen determinations were made by the urease aeration method of Meyers ('24). Urease was prepared by the method of Koch ('26).

TABLE 1

S.D.A. of glycine (controls). Ten grams glycine

DOG NO.	DATE	WEIGHT IN KILOGRAMS	AVERAGE B.M.R./HOUR, CALORIES	BASAL R.Q.	R.Q. AFTER GLYCINE	TOTAL CALORIES AFTER GLYCINE	TOTAL S.D.A., CALORIES	BLOOD UREA N BEFORE AMYTAL	BLOOD UREA N BEFORE GLYCINE	BLOOD UREA N AT END	EXCESS UREA UREA N	CALORIES/MILLIMOL GLYCINE DEAMINIZED
3	6/21/35	5.9	10.09	0.781	0.829	78.59[1]	18.05	...	17.2	18.8	1088.3	0.23
32	10/28/35	6.1	14.69	0.722	0.788	83.10	9.65	10.7	11.1	16.3	939.9	0.15
31	10/25/35	7.5	15.68	0.779	0.836	89.75	10.35	15.4	8.1	9.1	259.7	0.45
4	6/25/35	8.4	19.57	0.783	0.800	136.33[1]	18.91	...	30.0	19.0		
29	10/22/35	9.9	12.47	0.813	0.825	70.56	9.69	9.4	9.5	15.2	600.4	0.23
28	10/18/35	11.3	16.88	0.816	0.801	94.26	9.82	11.8	10.7	8.3	573.1	0.24
Av.				0.782	0.813		12.75		14.4	14.5		0.22

[1] Six hours.

RESULTS

In table 1 we have summarized the results of the injection of 10 gm. of glycine into each of six dogs. In all except dog 28, where the R.Q. was already high, the R.Q. rose. Heat

production also was increased. We are unable to explain why the S.D.A. should be so high in dogs 3 and 4, but when calculated on the basis of millimols of glycine metabolized dog 3 shows a value of 0.23 Cal. per millimol which is close to the average of 0.22. Since dog 4 started with such a high blood urea nitrogen (30 mg. %) and at the end of the experiment showed a figure so much lower (19.0 mg. %), we were unable to calculate the increase heat production in Calories/millimol, with any degree of accuracy. The value of 0.45

TABLE 2

A typical experiment. Dog 42, weight 6.4 kg., 11/19/35

TIME	B.M.R. CALORIES/HOUR	R.Q.	BLOOD UREA N MG./PER CENT	URINE UREA N/HOUR MG.
9.35–9.55	13.72	0.710	7.6	
10.02–10.25	13.94	0.712		
10.32–10.52	13.98	0.731	6.0	68.6
Bilateral nephrectomy 11.04–11.13				
11.17–11.37	12.22	0.704		
11.43–12.03	12.63	0.734		
12.07–12.37	12.59	0.759	7.9	
7.88 gm. glycine intravenously 12.45–1.05				
1.10–2.05	16.10	0.798	16.1	
2.12–3.00	15.26	0.819	21.9	
3.07–4.02	14.94	0.778	27.5	
4.07–5.00	14.19	0.743	30.0	
5.05–6.00	13.04	0.751	36.9	

Average B.M.R. after nephrectomy 12.48 Cal.
S.D.A. of glycine 11.13 Cal.

Cal./millimol seen in dog 31 is probably somewhat higher than the real increase should be. During the control period the blood urea nitrogen fell from 15.4 mg. % to 8.1 mg. %, which resulted in the elimination of considerable urea nitrogen over and above that actually formed during the period. As the control values were deducted from the total excreted during the experimental period, our deduction in this case was undoubtedly too great.

Table 2 shows a typical nephrectomy experiment. The main points to be emphasized here are the constancy of the

basal metabolism under sodium amytal, both before and after nephrectomy; the curve of the increased heat production after the injection of glycine; the increase in the R.Q. after glycine; and the accumulation of urea nitrogen in the blood stream at a practically constant and uniform rate.

TABLE 3

S.D.A. of nephrectomized dogs (7.88 gm. glycine)

DOG NO.	DATE	WEIGHT OF DOG IN KILOGRAMS	AVERAGE B.M.R. BEFORE INJECTION, CALORIES/HOUR	AVERAGE R.Q. BEFORE INJECTION	AVERAGE R.Q. AFTER INJECTION	TOTAL CALORIES AFTER INJECTION	S.D.A., CALORIES	BLOOD UREA N BEFORE INJECTION	BLOOD UREA N AT END	BASAL URINE UREA N EXCRETION/HOUR
45	11/22/35	5.2	8.01	0.745	0.817	53.52	13.47	8.8	44.4	17.7
50	12/17/35	6.1	10.14	0.734	0.831	63.50	12.80	7.9	33.9	56.0
38	11/ 8/35	6.4	11.67	0.793	0.876	69.03	10.68	7.4	26.7	75.7
39	11/11/35	6.4	11.09	0.754	0.802	70.98	15.53	11.7	50.0	81.3
42	11/19/35	6.4	12.48	0.732	0.778	73.53	11.13	7.9	36.9	68.6
46	12/10/35	6.4	10.63	0.746	0.800	68.66	15.51	14.4	52.7	91.8
43	11/20/35	6.8	11.69	0.830	0.890	72.91	14.46	29.2	44.1	87.5
47	12/11/35	8.2	14.90	0.761	0.835	90.68	16.18	10.8	38.1	126.1
48	12/13/35	8.2	12.26	0.807	0.818	83.35	20.05	11.3	27.5	32.8
36	11/ 6/35	8.4	12.75	0.747	0.856	79.12	15.37	7.2	29.9	60.2
51	12/20/35	8.6	15.47	0.755	0.783	86.40	11.27	13.6	38.3	189.3
44	11/21/35	10.2	15.75	0.782	0.745	77.56[1]	15.33[1]	13.4	38.8	29.8
35	11/ 5/35	10.7	15.91	0.853	0.858	96.36	16.81	17.5	51.5	411.6
40	11/12/35	13.2	17.46	0.741	0.763	105.09	17.79	14.6	49.3	184.6
Av.				0.770	0.818		14.74	12.5	40.1	108.1
Controls—115 cc. 0.9% NaCl given instead of glycine										
53	12/24/35	5.5	9.92	0.804	0.815	29.63[2]	0.13	19.7	23.1	68.3
55	12/30/35	6.4	9.18	0.759	0.809	38.56[2]	1.84	15.3	18.4	56.4
54	12/27/35	7.5	11.58	0.707	0.730	48.50[1]	2.18	14.5	21.8	66.4
Av.				0.757	0.785		1.30	16.5	21.1	63.7

[1] Four hours.

[2] Three hours.

The summarized results of the injection of glycine into nephrectomized dogs are shown in table 3. There is again an increase in the R.Q. occurring in all dogs except dog 44. The specific dynamic action of glycine is, if anything, slightly

higher than in the control series. Blood urea nitrogen increased markedly averaging more than a threefold increase over the 5-hour period.

In the three dogs injected with only sodium chloride solution, heat production was but slightly increased. If one deducts this slight average increase from the value obtained in the nephrectomized dogs, the average net specific dynamic action of the glycine amounts to 13.44 Cal. in contrast to 12.75 Cal. for the control series.

DISCUSSION

Our results show that it is practicable to study the specific dynamic action of glycine in the dog anesthetized with sodium amytal. When expressed in Calories per millimol of glycine metabolized they are in accord with the values of Wilhelmj and Mann ('30); but in our experiments the amount of glycine metabolized was somewhat less. Since the specific dynamic action of glycine can be studied after sodium amytal it is possible to determine the effect of glycine immediately after nephrectomy. This is particularly desirable because it eliminates to a large degree any complication of results that might result with the large accumulation of urea or other metabolic end products. The results on the nephrectomized dogs receiving only 0.9% sodium chloride seem to us to be sufficient evidence that our results are not greatly complicated by these factors.

It seems worth while to point out that our results confirm those of Chambers and Lusk ('30) that the specific dynamic action of glycine is, at least to a large extent, independent of the size of the dogs used.

Space does not permit an adequate discussion of the various theories of specific dynamic action of amino acids. Borsook ('36) had adequately reviewed them and attempted to combine them into a theory which is in accordance with the known facts. Our results on the unoperated dogs agree with his ('35) in so far as the correlation between excess Calories and excess urinary nitrogen are concerned. We

believe he ('31 a, '31 b, '31 c) has much over estimated the role of the kidney in the specific dynamic action of amino acids. We do not intend to imply that the kidney does no work in the concentration of the urine or the excretion of the excess urea, but our results do show that this work is so small when compared with the total heat production as to be, for all practical purposes, unmeasurable even when accurate methods are used.

CONCLUSIONS

1. The specific dynamic action of glycine, when expressed on the basis of Calorie per millimol deaminized, is the same when determined under sodium amytal anesthesia as in the unanesthetized dog.

2. The specific dynamic action of glycine is independent of the size of the dog, at least within the limits of those used in this investigation.

3. The kidney is not responsible for any measurable amount of the specific dynamic action of glycine.

LITERATURE CITED

BORSOOK, H. 1935 The correlation between excess calories and excess urinary nitrogen in the specific dynamic action of proteins in animals. Proc. Nat. Acad. Sci., vol. 21, p. 492.

———— 1936 The specific dynamic action of protein and amino acids in animals. Biol. Rev., vol. 11, p. 147.

BORSOOK, H. AND H. M. WINEGARDEN 1931 a The work of the kidney in the production of urine. Proc. Nat. Acad. Sci., vol. 17, p. 3.

———— 1931 b The energy cost of the excretion of urine. Proc. Nat. Acad. Sci., vol. 17, p. 13.

———— 1931 c On the specific dynamic action of protein. Proc. Nat. Acad. Sci., vol. 17, p. 75.

CHAMBERS, W. H. AND G. LUSK 1930 Animal calorimetry. Specific dynamic action in normal and phlorhizinized dogs. J. Biol. Chem., vol. 85, p. 611.

DOCK, W. 1931 The relative increase in metabolism of the liver and of other tissues during protein metabolism in the rat. Am. J. Physiol., vol. 97, p. 117.

EATON, A. G., S. C. CORDILL AND J. L. GOUAUX 1935 Is the work of the kidney, due to the excretion of urea, a factor in specific dynamic action? J. Nutrition, vol. 10, p. 429.

KOCH, F. C. 1926 Stable and convenient urease reagent and modified blood urea method. J. Lab. Clin. Med., vol. 11, p. 776.

MEYERS, V. 1924 Practical chemical analysis of the blood, 2nd ed.

STRIECK, F. 1928 Untersuchungen uber den Gesamtstoffwechsel und die spezifisch-dynamische Eiweisswirkung bei nephrektomierten Hunden. Arch. f. Exp. Path. u. Pharmakol., Bd. 136, S. 349.

WILHELMJ, C. M., J. L. BOLLMAN AND F. C. MANN 1928 Studies on physiology of liver; effect of removal of liver on specific dynamic action of amino acids administered intravenously. Am. J. Physiol., vol. 87, p. 497.

WILHELMJ, C. M. AND F. C. MANN 1930 The influence of nutrition on the response to certain amino acids. I. The effect of fasting. Am. J. Physiol., vol. 93, p. 69.

PHOSPHORUS DEFICIENCY METABOLISM AND FOOD UTILIZATION IN BEEF HEIFERS [1]

MAX KLEIBER, HAROLD GOSS AND HAROLD R. GUILBERT
Division of Animal Husbandry, College of Agriculture, University of California, Davis

(Received for publication April 4, 1936)

1. INTRODUCTION

The respiration trials reported in this paper were carried out in order to determine the influence of phosphorus deficiency on the efficiency of food utilization. The term 'efficiency of food utilization' may have several different meanings and is often used rather vaguely. It is necessary therefore to define the term.

Weight efficiency relates gain in body weight to weight of the food consumed. In this paper we are mainly dealing with energy efficiency, a relation between the chemical energy of the animals' products and the chemical energy in the food. By energy in the animals' product, or net energy N, we mean the heat of combustion of the gained body substance (or the heat of combustion of the produced milk plus the heat of combustion of the gained body substance etc.). The net energy is expressed in kilogram calories or in therms (1 therm = 1000 kg. calories.

The food energy may be expressed as heat of combustion of the food, as digestible energy or as metabolizable energy. The following formulation is applicable to any of these three bases for measuring food energy. The calculations of our

[1] This report is part of an investigation on the relation of nutrition to reproduction which became cooperative with the United States Bureau of Animal Industry, July 1, 1929.

results (tables 8, 9, 10) are based on metabolizable energy but table 5 furnishes the data necessary for expressing the results also in terms of digestible food energy or heat of combustion of the food. The unit for the food energy is the same as that for the net energy.

Two main types of efficiency should be distinguished, the total efficiency and the partial efficiency.

The total efficiency is the net energy per unit of the energy (heat of combustion or digestible energy or metabolizable energy) in the total food, thus:

$$\text{Total efficiency} = \frac{\text{Net energy}}{\text{energy in total food}} = \frac{N}{U} \qquad (1)$$

At a certain food level (maintenance level) the net energy will be zero, $N = 0$ (that does not mean that nothing is produced but that the sum of the energy in the product is zero, a milk cow, for example, may continue to yield milk but at the cost of her own body substance). The total efficiency at the maintenance level is zero. If the food ration is smaller than the maintenance requirement, the net energy, and consequently also the total efficiency are negative. The total efficiency characterizes the economy of animal production. It does not characterize the nutritive value of the food. In order to measure the food value it is necessary to eliminate the influence of the maintenance requirement of the animal. This can be done by feeding the animal at two different levels and determining the difference in net energy resulting from the difference in the food energy; thus one measures the effect of a part of the food energy and may formulate:

$$\text{Partial efficiency} = \frac{\text{Difference in net energy}}{\text{Difference in food energy}} = \frac{N_1 - N_2}{U_1 - U_2} \qquad (2)$$

Below maintenance both N_1 and N_2 will be negative and the partial efficiency will, as a rule, be positive even though the total efficiency is negative.

If the partial efficiency is independent of the plane of nutrition $\left(\frac{N_1 - N_2}{U_1 - U_2} = k\right)$ then the net energy N will be proportional to the difference of the total food energy U and the maintenance requirement M.

$$N = k\,(U - M) \tag{3}$$

In this case the following equation shows the relation between partial efficiency k and total efficiency $\frac{N}{U}$.

$$\frac{N}{U} = k\left(1 - \frac{M}{U}\right) \tag{4}$$

In order to formulate the relation of the total efficiency to the fasting katabolism B instead of the maintenance requirement M it may be considered that for maintenance $U_1 = M$ and $N_1 = 0$ and for fasting $U_2 = 0$ and $N_2 = -B$

$$k = \frac{N_1 - N_2}{U_1 - U_2} = \frac{0 - (-B)}{M - 0} = \frac{B}{M} \tag{5}$$

If this result $\left(M = \frac{B}{k}\right)$ is introduced into equation (4) one obtains for the total efficiency

$$\frac{N}{U} = k - \frac{B}{U} \tag{6}$$

The result of this equation may be stated as follows:

If the partial efficiency is independent of the plane of nutrition then the total efficiency is the difference between the partial efficiency and the quotient $\frac{\text{Fasting katabolism}}{\text{Total energy intake}}$.

The reciprocal of this quotient, namely $\left(\frac{U}{B}\right)$, is a measure for the level of food intake (relative food level) indicating how many times as much energy the animal takes in as it spends during fasting. If the animal can eat as much as it wants then the quotient $\frac{U_a}{B}$ characterizes the appetite of the animal.

The presupposition of a constant partial efficiency is strictly applicable only in special cases. The general formulation of the total efficiency (with variable partial efficiency)

is more complicated.[2] The definition of the terms by equation (6) is, however, sufficiently precise for the discussion in this paper.

An influence of phosphorus deficiency on the efficiency of food utilization has been observed by Theiler in his classical investigation on aphosphorosis in South Africa. Theiler ('33) concluded that animals given sufficient phosphorus utilize their food better and gain more per unit of food consumed than do animals on a phosphorus-low diet. Theiler thus observed that phosphorus deficiency lowers the total weight efficiency of the animals.

Eckles and Gullickson ('27) concluded from their experiments that cows on a phosphorus-low diet needed at least 20% more digestible nutrients to maintain their live weight than was indicated by Morrison's feeding standard.

Riddell, Hughes and Fitch ('34) studied the oxygen consumption of cows kept alternately at normal and low phosphorus intake. From the 6-minute records, obtained by

[2] In general the partial efficiency varies with variations in the plane of nutrition. It is often higher for submaintenance than for super-maintenance feeding. If k_p stands for the partial efficiency above maintenance and k_m for the partial efficiency below maintenance then the total efficiency may be expressed as follows:

$$\frac{N}{U} = k_p - \frac{k_m B}{k_m U} \qquad (6')$$

If the partial efficiency varies even within the submaintenance and supermaintenance levels as indicated by the result of Forbes et al. ('28), Wiegner and Ghoneim ('30), Mitchell et al. ('32) and confirmed by the experiments reported in this paper (p. 150) then the best characteristic for the nutritive value of the food would be the differential efficiency $\frac{dN}{dU} = \eta$ and in this case the total efficiency may be formulated as follows:

$$\frac{N}{U} = \frac{\int_0^U \eta \, dU}{U} - \frac{B}{U} \qquad (6'')$$

The term $\left(\frac{\int_0^U \eta \, dU}{U}\right)$ may be classified as the mean partial efficiency for the entire food level and the result summarized as follows:

In general the total efficiency is the difference between the mean partial efficiency of the entire food and the quotient $\frac{\text{Fasting katabolism}}{\text{Total energy intake}}$.

Brody's muzzle method 12 hours after the last food was given, they selected those that were most uniform and showed the lowest oxygen consumption. These authors concluded that the addition of phosphorus to a phosphorus-low diet lowers the general metabolism. The main criticism applying to their result is that the metabolism of cows 12 hours after the last feeding is neither a true fasting katabolism nor representative of the average daily metabolism of the fed animal.

The results reported in the present paper are based on thirty-four complete metabolism trials conducted with three pairs of beef heifers fed all the same diet low in phosphorus but one heifer of each pair receiving a supplement of phosphate. Each metabolism trial lasted 2 weeks. The fasting katabolism was determined in sixteen respiration trials of 48 hours duration during the fourth and fifth day of fasting.

The results of our experiments confirm the observation of previous investigators that phosphorus deficiency lowers the efficiency of food utilization for gain in weight, more specifically the total weight efficiency. The new information supplied by our work is the influence of phosphorus deficiency on the gain or loss of body substance in terms of chemical energy, the energy efficiency, and the differentiation of the total energy efficiency in the two components: partial efficiency and appetite, the appetite being determined as the quotient

$$\frac{\text{Intake of food energy at abundant feeding}}{\text{fasting katabolism}}.$$

2. METHOD

Animals used. Six beef heifers were selected and matched into pairs, their weights, their gains from birth, and also the weights of their ancestors being considered. Animals 18, 27 and 29 were pure bred Herefords; nos. 10 and 51 were pure bred Aberdeen-Angus; and no. 32 was a crossbred Aberdeen-Angus-Shorthorn. All the animals had been raised in the university herd. Only four were kept under observation throughout the entire experimental period. The animals were 14 to 15 months of age when started on the experiment.

Food and feeding. The guiding principle in selecting rations was to secure one deficient in phosphorus, yet complete with regard to other nutrient constituents. This is accomplished only with difficulty, particularly in providing adequate protein, when a roughage good in protein and low in phosphorus is not available. The rations used, together with the chemical composition, are shown in table 1.

TABLE 1

Composition of food per 100 gm. as fed

	RATION 1 CONTROL	RATION 2 LOW-PHOSPHORUS	RATION 3 CONTROL	RATION 4 LOW-PHOSPHORUS	RATION 5 CONTROL	RATION 6 LOW-PHOSPHORUS
	gm.	gm.	gm.	gm.	gm.	gm.
a. Food mixture						
Alfalfa molasses meal	30.0	30.0	30.0	30.0		
Dried beet pulp	33.0	30.0	30.0	30.0	62.0	62.5
Cornstarch	25.0	25.6	31.9	32.6	30.0	30.0
Casein	2.5	2.5	1.5	1.5	1.5	1.5
Corn gluten meal	7.5	7.5				
Cottonseed oil	1.5	1.5	3.0	3.0	2.5	2.5
Cod liver oil	0.5	0.5	0.5	0.5	1.0	1.0
Calcium carbonate	1.0	2.4	1.0	2.4	1.0	2.5
Calcium acid phosphate	2.0		2.1		2.0	
b. Chemical analyses						
Moisture	10.20	10.00	13.20	12.20	13.40	13.20
Crude protein	13.38	13.97	8.27	8.84	6.43	6.45
Fat	1.75	1.79	2.53	2.57	0.75	0.80
Ash	6.08	5.12	6.17	5.62	4.88	4.30
Crude fiber	11.72	12.06	11.80	11.05	9.94	10.54
N. F. E.	56.87	57.06	58.03	59.72	64.60	65.31
Calcium	1.57	1.43	1.48	1.52	1.28	1.29
Phosphorus	0.51	0.13	0.45	0.09	0.41	0.068
Carbon	37.68	38.20	38.40	38.00	38.20	39.20
Calories per gram	3.88	3.92	3.78	3.79	3.75	3.68

Cottonseed oil was used in the rations to reduce the dryness and dustiness caused principally by the cornstarch and to make the physical characteristics of the rations acceptable to the animals. The oils were first mixed with the beet pulp or beet pulp and alfalfa meal in a power mixer; then the cornstarch and other ingredients were added and mixed. The finer components adhered to the alfalfa meal and to the beet pulp so that the mixture remained uniform.

All animals were started on ration 1 on January 16, 1932, and continued until February 27, 1932, when no. 32 was changed to ration 2 (low in phosphorus). The remaining five animals continued on ration 1 throughout respiration trials 1 to 6 inclusive. Animals 51 and 27 were changed to low-phosphorus ration 2 on May 14th and May 28th, respectively, and were on this ration during respiration trials 7 to 9, inclusive. During July and August, 1932, control animals 10, 18 and 29 were changed to ration 3, and the low-phosphorus animals 27, 32 and 51 were changed to ration 4, the animals being on these rations during respiration trials 13 to 16, inclusive, and remaining until September 27, 1933. On this date heifers 18 and 29 were changed to control ration 5, and heifers 27 and 32 to phosphorus-low ration 6. The animals remained on these rations until July 26, 1934, when they were slaughtered. Shortly after nos. 18 and 29 were changed to ration 5, a monocalcium phosphate especially prepared for animal feeding was substituted for dicalcium phosphate. Soon thereafter the animals went off feed and did very poorly for about 6 weeks. It was discovered that something in the mineral supplement, presumably ferrous compounds, caused the fats in the ration to become rancid within 24 hours after mixing. As soon as dicalcium phosphate feeding was resumed, the appetite of the animals returned to normal.

Animals 10 and 51 were taken out of the experiment on July 18 and September 28, 1933, respectively.

The animals were fed individually twice each day during most of the experimental period. Feeding thrice daily was tried for a time to see whether or not a significantly higher feed intake could be attained. Except at feeding time during the early part of the experiment the animals all ran together in a small paved corral adjacent to their stalls. During the latter part of the experiment the controls were entirely separated from the low-phosphorus animals to prevent the latter from consuming phosphorus-rich feces of the controls.

The amounts of dry matter consumed per day during each metabolism trial are presented in table 5. During the first

nine trials the two pair mates were fed equal amounts of food at approximately one-half, one and one and one-half maintenance levels. After 1 year of experiment the animals on the P deficient diet would not even eat enough for maintenance (trials 13 to 16) equal amounts of food were still fed to the pair mates at the one-half maintenance level.

After 2 years of the P-low regimen the difference in weight between the animals of one pair became so great that equal amounts of food for both no longer represented the same food level. Food levels are not comparable when they are expressed in kilograms or calories per animal or per kilogram of the animals' weight but are comparable when given in terms of the energy requirement (for example one-half maintenance, maintenance, etc.). The maintenance requirement is approximately proportional to the fasting katabolism of the animal (see equation (5), p. 123) consequently the food level may also be expressed in terms of the fasting katabolism (equation (6)). The fasting katabolism in turn is proportional to the $\frac{3}{4}$ power of body weight (Kleiber, '32), therefore the food level may also be expressed in terms of the $\frac{3}{4}$ power of body weight and two animals on the same diet may be regarded as being on the same relative food level if their food intakes per kg.$^{\frac{3}{4}}$ are equal.[3] Based on this consideration we made the rations in trials 22 and 23 so that the pair mates were given approximately the same amount of food per kg.$^{\frac{3}{4}}$. The higher rations were measured according to the same principle but the phosphorus deficient animals ate only a fraction of these rations.

Methods of analysis. Composite samples of the feed used were taken for chemical analysis from a large batch of the mixed feed as the daily rations were being weighed out for the respiration trials. These composites were then run through a Wiley mill, reduced in size by quartering, and finally ground to pass a 40-mesh sieve. In the first six trials the total feces voided each day were dried in a large

[3] The symbol kg.$^{\frac{3}{4}}$ is used throughout this paper as unit of the $\frac{3}{4}$ power of body weight, $W^{\frac{3}{4}}$, where W is expressed in kilograms.

electric oven at 90° to 95°C. The dry residues were weighed and added together until the end of the 12-day trial. The total dry matter voided was thus determined. The dry feces were then run through a laboratory hammer mill, and samples obtained for chemical analysis.

When, later, it was found that this method of drying feces caused a loss of some carbon and nitrogen, a different procedure was adopted. The moist feces from each 24 hours were carefully mixed, and an aliquot was taken for total nitrogen and for moisture and volatile carbon compounds. This procedure has been described by Kleiber, Caldwell and Johnson ('36). The remaining fresh feces were then dried as before in the air oven at 70°C. and a composite dry sample was prepared for analysis. Corrections were then applied for losses of C during drying. The value used for the total nitrogen in the feces was that obtained from the analysis of the wet feces samples taken daily.

All feed and feces samples were analyzed for crude protein, fat, ash, crude fiber and moisture, according to Methods of Analysis, Association of Official Agricultural Chemists, 1930 edition. Calcium was determined on the ash by McCrudden's method ('10, '11). Phosphorus was determined colorimetrically by the method of Fiske and Subarrow ('25), modified for ash materials. The accuracy of this method for feed and feces phosphorus was repeatedly checked by the official volumetric method of the Association of Agricultural Chemists ('30).

Blood calcium was determined by the Clark-Collip ('25) method, and inorganic serum phosphate by the Fiske and Subarrow ('25) method.

The fuel value of feeds and feces was determined in an Emerson fuel calorimeter. Carbon was determined after each combustion by allowing the CO_2 to escape from the bomb through an absorbing tower containing $BaCl_2$—Ba $(OH)_2$ solution. Titration of the excess $Ba(OH)_2$ was made with a mixed indicator, described in detail by Kleiber ('35).

Carbon determination on urine was carried out by a wet-combustion process as described by Mohlman and Edwards ('31). Instead of using a mixture of H_3PO_4 and H_2SO_4 and a solution of chromic acid, a mixture of $K_2Cr_2O_7$ and NaH_2PO_4 was made up. The proportions used were 7 gm. $K_2Cr_2O_7$ to 25 gm. NaH_2PO_4. These amounts are the quantities used per determination of approximately 100 mg. carbon. In place of the mixture of H_3PO_4 and H_2SO_4, 90 cc. of concentrated H_2SO_4 were added. The method was tried on benzoic acid, sulphanilic acid and sodium formate. It produced 98.0, 99.1 and 99.0% recovery of the C, respectively.

The analyses of the bones for $CaCO_3$ were carried out on samples of dry, fat-free bones; for the determination of Ca and phosphorus the bone samples were ashed.

Respiration trials: Procedure. In order to determine the partial efficiency of energy utilization one must determine the energy balance at two different levels of food intake (see equation (2) of introduction). One of these levels may be at complete fasting. Earlier observations of Forbes and co-workers ('28) as well as Mitchell and collaborators ('32) have indicated that the partial efficiency of energy utilization in cattle may depend on the food level. It seemed desirable, therefore, to run trials with food levels below as well as above maintenance. The appetite of our animals on phosphorus-low rations, however, was so poor that the plan of studying the energy utilization at high food levels had to be abandoned. We have not even been successful in every case in keeping the low-phosphorus animals at a maintenance level.

The history of the 2½-year series of respiration trials with the beef heifers is shown in table 5. Each respiration trial for the animals except those on basal metabolism studies lasted 2 weeks, of which time two 5-day periods constituted uninterrupted respiration experiments. The average coefficient of variation of the daily O_2 consumption within one trial amounted to $\pm(4.4 \pm 0.8)\%$ for the phosphorus deficient and $\pm(4.2 \pm 0.6)\%$ for the control heifers. The corresponding figures for the CO_2 production are $\pm(4.5 \pm 0.91)\%$ for

the phosphorus deficient and $\pm(1.8 \pm 0.25)\%$ for the control heifers. The average standard deviation of the daily R.Q. within one trial was $\pm(0.029 \pm 0.006)$ for the phosphorus deficient and $\pm(0.028 \pm 0.007)$ for the control heifers. The measurements of the fasting katabolism were made in a 48-hour experiment during the fourth and fifth day after the last food.

The technic of the respiration trials followed at this station has been described in detail by Kleiber ('35).

3. SYMPTOMS OF PHOSPHORUS DEFICIENCY

Gain in weight. The weight curves of the animals essentially confirm the earlier observations concerning the effect of phosphorus deficiency on growth. During the first 6 months after changing to phosphorus-low rations 2 and 4, heifers 27, 32 and 51 continued to gain at nearly the same rate as their controls on rations 1 and 3. At the end of this time the low-phosphorus heifers ceased to gain; their weights remained about constant for a period of approximately a year. During this time the controls continued to gain. After the change to rations 5 and 6 the control animals maintained their weight while the weight of the low-phosphorus animals declined. In the following discussion the period of the first 6 months (apparently no influence of phosphorus-deficient food on growth) is termed the first period of the experiment. The following year (cessation of growth in phosphorus-deficient animals) is classified as the second, and the last half-year (decline in weight of the phosphorus-deficient animals) as the third period of the experiment. The difference between the initial and final weight of control animal 18 was 203 kg. that of control animal 29 was 190 kg., while the corresponding differences between initial and final weights of the phosphorus-deficient animals 32 and 27, was only 36 and 6 kg., respectively. Some evidence of limitation of skeletal growth is afforded by the fact that control animals 18 and 29 increased in height at withers 9.5 and 11 cm., respectively, while their low-phosphorus mates increased but 6.5 and 8.5 cm. in height, respectively.

Concentration of phosphorus in the blood serum. The data on serum phosphorus are presented in table 2. Most of the data are based upon composite samples collected on each of 2 or 3 successive days. A few determinations based upon single samples are included. The control animals maintained high inorganic phosphorus content of the blood serum

TABLE 2

Content of inorganic phosphorus in blood serum[1]

DATE	MILLIGRAMS PHOSPHORUS PER 100 CC. BLOOD SERUM					
	Control heifers			Low-phosphorus heifers		
	No. 10	No. 29	No. 18	No. 32	No. 27	No. 51
2–25–32	8.9 (1)	(1)	10.1 (1)	9.02 (1)	(1)	9.6 (1)
3–12–32	8.4	8.3		6.1 (2)	11.1	
3–19–32	7.9			6.8		
3–26–32	6.7		9.4	6.0		8.1
4– 2–32	9.1			7.0		
4– 7–32	9.3	9.2		6.7	10.4	
4–28–32			10.0			8.8
5–12–32	7.9	8.6		6.1	8.8 (2)	
6– 3–32	8.2			4.7		
6– 9–32	8.2		10.3	7.8		7.2 (2)
6–18–32	9.2			8.2		
7– 6–32	10.9 (3)	9.7 (3)	11.2 (3)	7.8 (4)	6.2 (4)	7.5 (4)
7–23–32	7.6	9.8	10.5	6.5	7.4	7.4
8– 6–32	7.9			7.3		
8–20–32	8.1	8.4	11.2	5.7	5.6	7.2
9– 2–32				4.4	4.2	5.1
9–10–32	7.8	8.7		5.1	5.5	5.5
9–17–32	8.4	9.3	8.2	6.5	4.7	6.6
10– 6–32	6.7	8.6	9.8	5.1	4.8	5.7
11–17–32	8.8	8.9	10.3	4.6	4.4	6.9
1–12–33	7.8	8.0	9.6	5.5	3.9	5.6
3–25–33	7.5	8.6	10.0	4.3	4.5	5.3
4–15–33	8.3	8.9	9.7	4.8	4.2	5.1
6–16–33		8.5	9.5	5.1	4.9	4.9
9–27–33		(5)	(5)	3.6 (6)	3.1 (6)	
12–19–33				4.6	4.4	
2–12–34		6.9	8.6	2.3	2.8	
3–24–34		7.9	8.7	3.5	3.7	
5–11–34		6.4	8.1	5.3	4.8	
7–25–34		9.4	11.3	3.5	3.9	
Average	8.27	8.56	9.79			

[1] The figures in parenthesis in the table indicate the ration fed and the date on which the change in ration was made.

throughout the experiment, the range being from 6.4 to 11.3 mg. phosphorus per 100 cc. of serum. Considering the age of the animals, the average values of 8.27, 8.57 and 9.8 mg. phosphorus per 100 cc. serum for nos. 10, 29 and 18, respectively, are somewhat higher than the 'normal' figures usually found (Palmer, Cunningham and Eckles, '30), (Guilbert, unpublished data) and presumably reflect the high phosphorus content of the control rations.

After the change from ration 1 to phosphorus-low ration 2, the blood phosphorus of animal 32 dropped from 9 to an average of 6.77 mg. phosphorus per 100 cc. of serum; after the change to ration 4 the average value was 5.42 mg. phosphorus; and during the period of ration 6 the average value dropped to 3.83 mg. phosphorus per 100 cc. The average values for animal 27 during the periods on rations 4 and 6 were 5.05 mg. phosphorus and 3.95 mg. phosphorus per 100 cc. of serum, respectively. The values for animal 51 during the period on ration 4 averaged considerably higher than those for the other animals on the same feed. The consistently high values found for control animal 18 are also noteworthy.

Although the serum phosphorus values decreased in relation to the phosphorus content of the rations fed, only a few of the determinations during the latter part of the experiment would be indicative of phosphorus deficiency on the basis of the criteria of Palmer, Cunningham and Eckles ('30). The vitamin D from the cod liver oil fed to our animals throughout the experiment may have prevented a more conspicuous fall in blood phosphorus. The range in blood-phosphorus values of dairy heifers on phosphorus-low rations reported by Van Landingham, Henderson and Bowling ('35) are somewhat similar to ours.

Pica. All the low-phosphorus animals began showing depraved appetites about December, 1932, coincident with cessation of increase in weight. It was manifested largely by coprophagy, chewing mangers and the chains by which the animals were tied at feeding time. After coprophagy started,

the stalls and corral were swept three times daily and washed with water frequently to prevent the accumulation of dried feces from the control animals, from which the low-phosphorus animals could obtain a significant addition to their phosphorus intake. Notwithstanding these precautions the low-phosphorus animals may at times have obtained added phosphorus in this manner, and this accident may have caused some of the variations found in the serum phosphorus values.

By April, 1933, heifers 32 and 27 showed marked osteophagia when tested with sun-bleached bones. Animal 51 at this time manifested only mild interest, while none of the controls would touch the bones. At this time the serum phosphorus was from 4.20 to over 5.0 mg. phosphorus per 100 cc. of serum. Heifer 51 exhibited definite osteophagia in August, 1933. Animals 32 and 27 were then still in good and animal 51 in excellent condition. As the experiment progressed, pica became more pronounced. All the low-phosphorus animals eagerly licked up blood spilled on the cement floor when blood samples were being taken.

No depraved appetite was noted in the control animals until after the change to ration 5. Wood chewing and occasional licking of urine from the concrete pavement were noted. In other experiments at this station wood chewing has been observed in connection with 'no-roughage' rations regardless of the completeness of the ration in other respects. The licking of urine associated with low-protein intake, has been observed also in other experiments by one of us (H. R. Guilbert).

Lack of appetite. Lack of appetite described by earlier workers (see for example, Huffman, Duncan, Robinson and Lamb, '33) was one of the most conspicuous symptoms in our phosphorus deficient animals.

Table 3 shows the maximum of the weekly food intake per kg.$^{\frac{3}{4}}$ during the preliminary period when all animals received a phosphate supplement and during each of the subsequent periods when animals 27 and 32 were on phosphorus-low rations. The reason for expressing the appetite as food intake per unit of the $\frac{3}{4}$ power of weight has been discussed before (p. 128).

The phosphorus-deficient animals decreased the maximal food intake per kg.$^{\frac{3}{4}}$ consistently as the phosphorus in the food decreased. From the first to the second period the low-phosphorus animals lost about one-fifth of their appetite, while the appetite of the control animals increased slightly.

During the last period of the experiment the maximal food intake per kg.$^{\frac{3}{4}}$ of the two low-phosphorus animals approximated a level one-half of that of the first period. Only a small part of this drastic loss in appetite may be accounted for by a change in the composition of the food aside from its phosphorus content, as indicated by a 10% loss in appetite

TABLE 3

Appetite of phosphorus-deficient and control animals

ANIMALS	MAXIMAL INTAKE OF AIR-DRY FOOD PER WEEK PER UNIT OF BODY SIZE			
	Preliminary period	Time of phosphorus deficiency		
		0–6 months	6–18 months	18–24 months
	kg./w$^{\frac{3}{4}}$ [1]	kg./w$^{\frac{3}{4}}$	kg./w$^{\frac{3}{4}}$	kg./w$^{\frac{3}{4}}$
Phosphorus-deficient animals				
No. 27	0.437	0.470	0.404	0.260
No. 32	0.465	0.490	0.364	0.234
Control animals				
No. 29	0.481	0.467	0.526	0.367
No. 18	0.407	0.480	0.485	0.473

[1] Heading reads: "kg. of food divided by W to the $\frac{3}{4}$ power."

of the control animals. Thus our results confirm the observation of earlier workers (Theiler, Riddell) that phosphorus deficiency depresses the appetite of the animals.

Oestrus cycles. During the period January 15, 1932, to January 7, 1933 control animals 18 and 29 exhibited fifteen and sixteen periods of oestrus, respectively. During the next year and a half these animals were for the most part kept in separate quarters from the low-phosphorus animals and were not under so close observation as before. Moreover, they were not particularly demonstrative during oestrus, so that doubtless some periods were not recorded. Seven and nine periods, respectively, were recorded during this period. The occurrence of oestrus appeared somewhat irregular after change

to ration 5, in which the protein level was rather low. At autopsy, however, the ovaries of both animals were found to be normal, and they contained recent corpora. The third control animal, 10, had thirteen periods of oestrus during the first 9 months on the experiment. At the end of this time she bred, conceived, and in due time gave birth to a normal calf. This was considered to be evidence that the control rations 1 and 3 were reasonably adequate.

Animal 27 had, between January, 1931, and May, 1932, six periods of oestrus. At the end of this period she was changed to the phosphorus-low ration. Only three periods of oestrus were observed during the next 7 months, and only two additional periods during the next year and a half of the experiment. At autopsy she was found to have one small, quiescent ovary and one cystic ovary. Low-phosphorus animal 32 came into oestrus ten times during the first 9 months. At the end of this time (September 2, 1932) she was bred but did not become pregnant. A bloody discharge from the vagina was noted on January 21, 1933, and oestrus was recorded on October 18, 1933 and January 3, 1934. These three occasions were the only manifestations of oestrus during the entire period of the experiment, after breeding on September 2, 1932. On autopsy the ovaries of this animal were found to be small and quiescent. The cessation of oestrus in both these low-phosphorus animals coincided closely with cessation of gains. Low-phosphorus animal 51 continued to have more or less regular cycles throughout the entire period she was on the experiment. This animal always had a more thrifty appearance than 27 and 32, a better appetite, a higher content of inorganic phosphate in the blood serum. She was removed from the experiment in September, 1933, and was never placed on phosphorus low ration 6.

Cessation or irregularity of oestrus has been associated with phosphorus deficiency by a number of workers. The literature was reviewed by Eckles, Palmer and associates ('35). The experiments reported in their paper cast some doubt as to whether the interference with oestrus is caused

by phosphorus deficiency per se, as their animals on phosphorus-low rations had normal oestrous cycles. From the standpoint of blood and bone analysis and other symptoms, their animals were deficient in phosphorus; but apparently the intake of phosphorus was varied so that total food consumption was not significantly reduced.

Bone analysis. According to Neal and Palmer ('31) the ribs may be considered as the most reliable single samples that represent the condition of the skeleton with regard to its chemical composition. The analysis of the dry, fat-free rib bones of our heifers is presented in table 4.

TABLE 4

Composition of rib bone of phosphorus-deficient and control heifers

CONSTITUENTS	CONTENT PER 100 GM. OF FAT FREE DRY BONES			
	Phosphorus-deficient heifers		Control heifers	
	No. 27	No. 32	No. 18	No. 39
Ash	55.4	58.1	63.0	65.4
Ca	21.6	22.5	24.1	25.0
P	9.49	10.05	11.32	11.75
Mg	0.29	0.32	0.54	0.48
$CaCO_3$	8.25	8.25	6.15	7.00
$Ca_3(PO_4)_2$	46.20	48.90	54.30	56.70
Ratio: $\frac{Ca_3(PO_4)_2}{CaCO_3}$	5.6	5.9	8.8	8.1

The bones of the phosphorus-deficient heifers 27 and 32 had a lower ash content and a higher content of $CaCO_3$ than the bones of their mates, 18 and 29, on the phosphate-supplemented diet. The ratio $Ca_3(PO_4)_2 : CaCO_3$ is considerably lower in the bones of the control heifers. This result confirms the earlier findings of Neal, Palmer, Eckles and Gullickson ('31). In our trials the effect seemed somewhat more pronounced than in the trials of these authors.

4. RESULTS OF METABOLISM TRIALS

The results of the respiration trials carried out with the six beef heifers are summarized in chronological order in table 5.

TABLE 5

Phosphorus-deficiency and metabolism chronological summary

TRIAL NO.	DATE	ANIMAL NO.	WEIGHT OF ANIMAL	W$^{\frac{3}{4}}$ kg.$^{\frac{3}{4}}$	FEED EATEN IN GRAMS DRY MATTER PER DAY	P LEVEL: GRAMS P PER 100 GM. DRY FOOD	P BALANCE: GRAMS P PER DAY	DAILY ENERGY BALANCE (THERMS)				
								Feed	Digested	Metabolizable	Net	Heat
	1932		*kg.*									
1	Feb. 29 to Mar. 12	18 51	317.1 308.0	75.0 73.5	1207 1207	0.608 0.608	+ 2.08 + 3.81	5.27 5.27	4.14 4.19	3.08 3.48	— 2.57 — 2.78	5.65 6.31
2	Mar. 14 to Mar. 26	29 27	323.4 362.4	76.0 83.5	1207 1207	0.608 0.608	+ 1.78 — 0.77	5.27 5.27	3.97 4.08	3.04 2.97	— 3.69 — 3.83	6.73 6.80
3	Mar. 28 to Apr. 9	18 51	313.4 315.7	74.0 74.0	2395 2395	0.576 0.621	+ 6.19 + 6.84	10.45 10.46	8.47 8.56	6.96 6.95	— 0.10 — 0.18	7.06 7.13
4	Apr. 11 to Apr. 23	29 27	327.0 360.6	77.0 83.0	2395 2395	0.576 0.621	+ 6.66 + 5.27	10.45 10.46	8.59 8.41	7.11 6.89	— 0.30 — 0.68	7.41 7.57
5	May 2 to May 14	18 51	341.1 328.4	79.0 77.5	3895 4100	0.579 0.592	+ 8.52 + 5.62	16.93 17.73	13.61 14.63	11.65 12.29	+ 3.08 + 3.29	8.57 9.00
6	May 16 to May 28	29 27	351.5 385.6	81.0 87.5	4100 4100	0.579 0.592	+ 5.91 + 8.21	17.76 17.73	14.21 14.19	12.34 12.33	+ 3.01 + 3.25	9.33 9.08
7	June 6 to June 18	10 32	338.8 354.3	79.0 82.0	4474 4425	0.535 0.137	+ 3.9 + 1.0	19.33 19.29	15.25 15.63	13.31 13.33	+ 1.05 + 1.72	12.26 11.61
8	July 5 to July 16	10 32	342.5 356.1	80.0 82.0	3720 3764	0.500 0.138	+ 3.3 + 0.3	10.76 10.90	8.81 8.83	7.27 7.29	— 0.36 — 0.46	7.63 7.75
9	July 25 to Aug. 6	10 32	328.9 347.9	77.0 80.8	1241 1239	0.497 0.124	+ 3.3 + 0.42	5.37 5.39	4.51 4.77	3.63 3.81	— 2.23 — 2.37	5.86 6.18
10	Dec. 16 to Dec. 18	18 51	453.6 427.7	93.0 94.0								6.59 8.26
11	Dec. 19 to Dec. 21	29 27	490.7 530.7	104.0 111.0								8.32 8.78
12	Dec. 22 to Dec. 24	10 32	415.0 406.0	92.0 91.0								6.78 6.59
	1933											

11	Dec. 19 to	29	490.7	104.0								
	Dec. 21	27	530.7	111.0								8.78
12	Dec. 22 to	10	415.0	92.0								6.78
	Dec. 24	32	466.0	91.0								6.58
	1933											
13	Mar. 20 to	29	521.2	109.0	4331	0.514	— 1.3	18.87	14.92	12.45	+ 2.06	10.39
	Apr. 1	27	523.0	110.0	2814	0.104	— 0.44	12.55	10.00	8.09	— 1.64	9.73
14	Apr. 17 to	10	474.9	102.0	4331	0.514	— 2.2	18.87	14.85	12.71	+ 1.88	10.83
	Apr. 29	32	414.6	92.0	2640	0.104	— 0.59	11.89	9.81	8.54	— 0.33	8.87
15	May 1 to	29	514.8	108.0	1600	0.571	+ 1.2	6.83	5.50	4.38	— 3.35	7.73
	May 13	27	505.3	107.0	1634	0.112	— 0.16	6.95	5.42	4.32	— 4.39	8.71
16	May 15 to	10	464.0	100.0	1600	0.571	+ 0.8	6.83	5.50	4.28	— 4.74	9.02
	May 27	32	416.4	92.0	1634	0.112	— 0.1	6.95	5.83	4.58	— 2.45	7.03
17	May 28 to	29	494.0	104.8								7.54
	May 29	27	493.5	104.7								7.36
18	May 30 to	18	517.1	108.4								8.41
	May 31	51	473.1	101.4								8.04
19	June 1 to	10	442.7	96.5								9.91
	June 2	32	396.4	88.9								5.89
	1934											
20	Mar. 12 to	29	565.2	116.0	2756	0.469	+ 0.18	11.62	9.56	8.31	— 1.09	9.40
	Mar. 24	27	445.4	97.0	1656	0.078	— 0.48	7.02	6.12	4.93	— 2.94	7.87
21	Mar. 26 to	18	561.1	115.0	2800	0.483	+ 0.88	11.77	9.49	8.29	— 0.50	8.79
	Apr. 7	32	382.4	86.0	1179	0.077	— 0.25	5.04	4.36	3.47	— 2.62	6.09
22	Apr. 9 to	29	535.3	111.0	1410	0.468	+ 0.59	5.97	5.09	4.15	— 3.80	7.95
	Apr. 21	27	430.5	94.0	1209	0.073	— 0.69	5.14	4.33	3.37	— 3.76	7.13
23	Apr. 23 to	18	529.0	110.0	1401	0.468	+ 1.47	5.93	4.72	3.62	— 4.30	7.92
	May 5	32	369.6	84.0	1203	0.074	— 0.52	5.11	4.33	3.44	— 2.30	5.74
24	May 7 to	29	521.2	109.1							— 8.561	7.916
	May 9	27	413.7	91.7							— 7.793	7.067
25	May 9 to	18	516.6	108.3							— 6.967	6.568
	May 11	32	355.9	81.9							— 5.650	5.189

Phosphorus deficiency and digestibility. The digestibility of the energy of the experimental food was high and uniform throughout the experiment. The standard deviation of the single experiment is $\pm 3.1\%$ for the deficient animals and $\pm 2.5\%$ for the controls. The average digestibility of the food energy in eight respiration trials with the phosphorus-deficient animals in the second and third periods of the experiment is $83 \pm 1.1\%$. The average digestibility of the food energy in the corresponding experiments in the control animals is $81 \pm 0.9\%$. No influence of phosphorus deficiency on the digestibility of protein could be observed. The apparent protein digestibility in the phosphorus-deficient animals was $46.1 \pm 5.6\%$; that of the control animals $46.4 \pm 2.2\%$.

Phosphorus deficiency and urine excretion. The phosphorus-deficient animals excreted on the average slightly less nitrogen in the urine per day than did the controls. The difference, which is insignificant, may be partly related to the somewhat higher food intake of the normal animals. In the fasting katabolism trials the urine was collected during the fourth and fifth day of fasting, and one-half the amount of the nitrogen excretion thus found was regarded as the daily nitrogen excretion during fasting. The daily nitrogen loss during fasting was higher than the nitrogen loss of the animals during food intake. The daily average nitrogen loss for the phosphorus-deficient animals during fasting was 34.5 gm. nitrogen. The normal animals lost on the average 38.1 gm. nitrogen per day of fasting. There was thus no increase in protein katabolism during fasting of the phosphorus-deficient animals as compared with their mates.

In the urine of the animals receiving food the $\frac{C}{N}$ ratio $\left(\frac{\text{grams C}}{\text{grams N}}\right)$ showed a tendency to be increased with increasing plane of nutrition, ranging from 1.5 to 2.4 in the urine of the phosphorus-deficient animals and from 1.3 to 2.6 in the urine of the controls. The average urinary $\frac{C}{N}$ ratio for the phosphorus-deficient animals is slightly below that of the controls. The difference is, however, negligible.

During fasting the $\frac{C}{N}$ ratio in the urine dropped to an average of 1.08 for both groups of heifers, a fact indicating that in starvation the composition of the urine of ruminants approaches that of carnivorous animals.

The phosphorus-deficient animals used the digested protein less efficiently for sparing body protein than the control animals. This result follows from the data in table 6, where the partial relative protein katabolism, namely the increase in urinary nitrogen divided by the corresponding increase in digested nitrogen, is calculated for the change from the one-

TABLE 6

Partial relative protein katabolism ($\frac{\Delta \text{ N in urine}}{\Delta \text{ N digested}}$) *for one-half maintenance to maintenance level*

TRIAL NO.	PHOSPHORUS-DEFICIENT ANIMALS						CONTROL ANIMALS					
	Animal	N digested	N in urine	Δ N digested	Δ N in urine	Δ N in urine / Δ N digested	Animal	N digested	N in urine	Δ N digested	Δ N in urine	Δ N in urine / Δ N digested
13	27	27.0	24.5	21.0	11.4	0.55	29	35.6	28.8	22.8	4.3	0.19
15	27	6.0	13.1				29	12.8	24.5			
14	32	26.9	22.2	10.8	5.9	0.55	10	32.0	20.7	18.6	—0.7	—0.04
16	32	16.1	16.3				10	13.4	21.4			
20	27	10.7	15.5	6.8	4.6	0.68	29	13.6	10.8	6.9	1.0	0.15
22	27	3.9	10.9				29	6.7	9.8			
21	32	6.3	10.6	2.1	2.0	0.95	18	11.9	12.7	5.8	0.6	0.10
23	32	4.2	8.6				18	6.1	12.1			

half maintenance to the maintenance food level. The average of this quotient for the second and third periods of phosphorus deficiency amounts to 0.68 ± 0.10 gm. nitrogen katabolized per gram nitrogen digested for the phosphorus-deficient animals and to only 0.10 ± 0.05 gm. nitrogen katabolized per gram nitrogen digested for the control animals.

CH_4 production. The amounts of CH_4 produced during the second and third periods of the experiment ranged from seventy-two to 161 liters per day for the control animals.

The rate of CH_4 production is strongly influenced by the plane of nutrition. The correlation coefficient for food intake

and CH_4 production amounts to 0.72 for the phosphorus-deficient and 0.92 for the control animals. The effect of food intake on CH_4 production in our trials may be summarized by the following regression equations for the phosphorus-deficient animals:

$$CH_4 = 37 + 32.6\,F$$

for the control animals:

$$CH_4 = 29 + 32.9\,F$$

where CH_4 = amount of CH_4 produced daily in liters at standard conditions, F = amount of dry matter of food consumed daily in kilograms.

The metabolizability of the food energy is the factor by which the amount of total food energy (heat of combustion as measured in the calorimetric bomb) is to be multiplied in order to calculate the amount of energy available for meat, fat or heat production. The average metabolizability of the food energy in the phosphorus-low rations of $67.0 \pm 1.1\%$ is practically equal to the average metabolizability of $66.6 \pm 1.3\%$ for the energy in the control rations.

CO_2 production. Table 7 presents the results on the respiratory exchange of the phosphorus deficient and the control animals during the second and third period of our experiment. The effect of phosphorus deficient food on the respiratory exchange should be compared with the effect of the phosphorus supplemented food. For this comparison it is necessary to summarize the effect of food on the respiratory exchange in all the trials with phosphorus deficient as well as in the trials with the control animals, since the original plan of having a comparison within each pair trial was defeated by the low appetite of the phosphorus-deficient animals. In order to summarize the effect of food intake on the respiratory exchange one may assume a linear relation between the two variables, such as formulated in equation (7)

$$y = y_0 + r\,x \qquad (7)$$

where y = respiratory exchange, y_0 = respiratory exchange without food, x = food intake, r = a constant characterizing the effect of food.

TABLE 7

Respiratory exchange of phosphorus-deficient and control animals

TRIAL NO.	PHOSPHORUS-DEFICIENT ANIMALS					CONTROL ANIMALS				
	Animal No.	Food dry consumed per day	CO_2 produced per day liters (s)[1]	O_2 consumed per day liters (s)	R. Q.	Animal No.	Food dry consumed per day	CO_2 produced per day liters (s)	O_2 consumed per day liters (s)	R. Q.
		gm.					*gm.*			
13	27	2814	1883 ± 29	2015 ± 30	0.958 ± 0.013	29	4331	2222 ± 14	2201 ± 45	1.042 ± 0.018
14	32	2640	1714 ± 40	1705 ± 44	1.013 ± 0.033	10	4331	2282 ± 10	2188 ± 32	1.035 ± 0.005
15	27	1634	1538 ± 24	1812 ± 27	0.871 ± 0.015	29	1600	1441 ± 11	1691 ± 24	0.874 ± 0.007
16	32	1634	1307 ± 25	1468 ± 40	0.915 ± 0.021	10	1600	1633 ± 12	1956 ± 36	0.871 ± 0.013
17	27	0	1129	1834	0.616	29	0	1128	1620	0.711
18	51	0	1237	1783	0.694	18	0	1259	1819	0.709
19	32	0	964	1284	0.704	10	0	1484	2170	0.700
20	27	1656	1513 ± 7	1648 ± 11	0.919 ± 0.007	29	2756	1880 ± 9	1960 ± 14	0.959 ± 0.003
21	32	1179	1158 ± 36	1247 ± 35	0.928 ± 0.013	18	2800	1819 ± 13	1891 ± 29	0.974 ± 0.006
22	27	1209	1310 ± 9	1516 ± 8	0.863 ± 0.003	29	1410	1452 ± 8	1697 ± 14	0.856 ± 0.004
23	32	1203	1100 ± 12	1222 ± 11	0.898 ± 0.003	18	1401	1442 ± 11	1630 ± 27	0.887 ± 0.016
24	27	0	1088	1536	0.708	29	0	1232	1730	0.712
25	32	0	888	1210	0.734	18	0	1075	1478	0.727

[1] Liters(s) = liters at 0°C., 760 mm. Hg pressure and dry.

If y_0 and r are constant they may be calculated from any number of experiments with varying x and y. The respiratory exchange without food, y_0, can, however, not be regarded as a constant since it depends on the body size of the animals, which varied during the experiment. In order to eliminate the influence of body size we divide the entire equation (7) by the $\frac{3}{4}$ power of body weight and formulate

$$\frac{y}{W^{\frac{3}{4}}} = \frac{y_0}{W^{\frac{3}{4}}} + r\,\frac{x}{W^{\frac{3}{4}}} \tag{8}$$

Since the fasting katabolism in calories per kg.$^{\frac{3}{4}}$ is constant and since the respiratory exchange at a given R. Q. is proportional to the heat production, the term $\frac{y_0}{W^{\frac{3}{4}}}$, the fasting gas exchange per kg.$^{\frac{3}{4}}$, may also be regarded as a constant. The method of least squares (Ezekiel, '30, p. 55) has thus been applied to the relation of gas exchange per kg.$^{\frac{3}{4}}$ and food consumption per kg.$^{\frac{3}{4}}$.

The following regression equation resulted from twelve respiration trials with the phosphorus-deficient animals (27 and 32) during the second and third periods of the experiment (table 7):

$$C = 10.7 + 0.248\,f \tag{9}$$

where C = liters CO_2 produced daily per kg.$^{\frac{3}{4}}$, f = grams dry matter of food consumed daily per kg.$^{\frac{3}{4}}$.

The corresponding equation for the two control animals 29 and 18 based on ten respiration trials reads

$$C = 10.6 + 0.233\,f \tag{10}$$

The difference in the regression coefficients seems to indicate that the food increases the katabolic processes in the phosphorus-deficient animals to a greater extent than in the control animals. This difference in the regression coefficients in our trials, namely 0.015 ± 0.0244 is, however, not statistically significant, the probability that it occurred by random being between 50 to 60% (Fisher, '30, table 14). To prove this difference according to statistical rule, 105 pair trials would have to be carried out if the standard deviation of a

determination would remain the same as in our experiments and if the mean results did not change.

O_2 consumption. The data for O_2 consumption during the second and third period of our experiment are presented in table 7. During the fourth day of fasting the phosphorus deficient animals consumed on the average daily 15.3 ± 0.71 liters O_2 per kg.$^{\frac{3}{4}}$. Practically the same result was observed with the control animals namely 15.4 ± 0.23 liters O_2 per kg.$^{\frac{3}{4}}$. In the averages for the phosphorus-deficient animals one result with animal 27 has been omitted since an R. Q. of only 0.62 showed that it was erroneous. The conclusion that phosphorus deficiency did not materially affect the O_2 consumption during fasting is, however, justified, whether or not this erroneous result is included in the calculation. It is interesting to note that animal 27 did not decrease its O_2 consumption when the food intake was reduced from the one-half maintenance level to fasting; it showed even a slight increase from 16.1 liters O_2 per day per kg.$^{\frac{3}{4}}$ at one-half maintenance to 16.8 liters O_2 per day per kg.$^{\frac{3}{4}}$ during fasting which, however, is statistically not significant.

Assuming a linear relation between O_2 consumption per kg.$^{\frac{3}{4}}$ and food intake per kg.$^{\frac{3}{4}}$ in order to give an approximate summary of our results (as explained for CO_2) the following regression equations have been calculated by the method of least squares applied to the data obtained in respiration trials 13 to 25: for phosphorus-deficient animals

$$O = 14.89 + 0.108\,f$$

For the control animals

$$O = 15.04 + 0.067\,f$$

The terms in these equations are defined as follows: $O =$ liters O_2 consumed per day per kg.$^{\frac{3}{4}}$. $f =$ grams dry matter of food eaten per day per kg$^{\frac{3}{4}}$.

The regression coefficients for O_2 consumption on food intake (0.108 and 0.067) are considerably lower than the regression coefficient for CO_2 production on food consumption (0.248 and 0.233). This result is to be expected if the food

contains mainly carbohydrates. If the animal has an R. Q. of 0.7 during fasting and subsequently raises the R. Q. to 1.0 by ingestion of carbohydrates, then, theoretically, the CO_2 production may increase 40% without a rise in the O_2 consumption.

The regression coefficient of O_2 consumption on food consumption is higher in the phosphorus-deficient animals than in the controls. This relation parallels that for CO_2. The difference is likewise not statistically significant. The probability that the difference is a matter of random is 20 to 30%. At least forty-nine additional pair trials with the same results as those discussed here would be necessary to prove with statistical significance the increase in the stimulating effect of food consumption on O_2 consumption as a result of phosphorus deficiency.

R. Q. During the second and third periods the R. Q. of the phosphorus-deficient animals varied from 0.708 to 1.013 (maximum food intake) (table 7). The corresponding range for the control animals was from 0.709 to 1.042.

The R. Q. of both groups of animals is essentially influenced by the plane of nutrition. The correlation coefficient of R. Q. and the food intake per kg.$^{\frac{3}{4}}$ amount to 0.96 for the phosphorus-deficient and to 0.98 for the control animals.

The linear regression equations calculated by the method of least squares are as follows:

For the phosphorus deficient animals

$$R.\,Q. = 0.731 + 0.10\,f$$

For the control animals

$$R.\,Q. = 0.730 + 0.009\,f$$

The term f stands, as in the previous equations, for grams dry matter of food consumed daily per kg.$^{\frac{3}{4}}$.

During the fourth day of fasting the average R. Q. of the phosphorus-deficient heifers amounted to 0.715 ± 0.009 (three trials); the R. Q. of the control animals to 0.716 ± 0.004 (four trials).

The results of the respiration trials during the second and third periods of the experiment lead thus to the conclusion

that phosphorus deficiency had no influence on the R.Q. of the fasting animals. The slight increase in the R. Q. of the phosphorus-deficient animals with food over that of the control animals is insignificant.

Phosphorus deficiency and fasting metabolism. The calorific value of CO_2 produced by our beef heifers during the fourth and the fifth day of fasting amounted to 6.52 kg. calories per liter. This result has been obtained by calculating the energy loss on the basis of the C and N balances in

TABLE 8

Heat increment and partial energy efficiency. Example of calculation

ANIMAL NO.	TRIAL NO.	FOOD ENERGY METABOLIZABLE PER DAY PER kg.$^{\frac{3}{4}}$ U	HEAT PRODUCED PER DAY PER kg.$^{\frac{3}{4}}$ Q	INCREASE IN METABOLIZABLE FOOD ENERGY ΔU	INCREASE IN HEAT PRODUCTION ΔQ	RELATIVE HEAT INCREMENT $\frac{\Delta Q}{\Delta U}$	PARTIAL ENERGY EFFICIENCY $1-\frac{\Delta Q}{\Delta U}$
Phosphorus deficient		*kg. cal.*	*kg. cal.*	*kg. cal.*	*kg. cal.*	*%*	*%*
27	17	0.0	67.8				
				40.3	13.6	33.7	66.3
	15	40.3	81.4				
				33.4	7.1	21.3	78.7
	13	73.7	88.5				
	24	0.0	77.1				
				35.8	— 1.2	— 3.4	103.4
	22	35.8	75.9				
				15.0	5.2	34.7	65.3
	20	50.8	81.1				

trials 24 and 25. If this figure is used in order to calculate the heat production of the animals during the fifth day of fasting in the second and third periods, one derives an average daily fasting katabolism per kg.$^{\frac{3}{4}}$ of (69.3 ± 3.0) kg. calories for the phosphorus-deficient animals (four trials) and (70.3 ± 5.0) kg. calories for the controls (three trials).

Calorigenic action of the food and partial energy efficiency. Table 8 gives an example of the calculation of the relative heat increment, that is, the heat increment or calorigenic action of the food in per cent of the metabolizable energy. The table also shows how the partial-energy efficiency is derived from the relative heat increment.

Fifteen out of twenty comparisons between different food levels in our trials confirm earlier observations of Forbes ('28) and Mitchell and co-workers ('32) that the calorigenic action per unit of food energy in cattle is increased with increasing plane of nutrition.

Thus in eight comparisons of heat production of our heifers at normal phosphorus intake there was an average calorigenic action of $11.8 \pm 3.4\%$ of metabolizable food energy for the change from fasting to one-half maintenance, while the corresponding calorigenic action in changing from one-half maintenance to full maintenance amounted to $26.6 \pm 3.9\%$.

Since the partial efficiency is thus affected by the plane of nutrition the comparison between the efficiency of phosphorus-deficient and control animals should be made at the same food level.

Two animals are assumed to be at the same food level if they take in equal amounts of metabolizable energy of a given food mixture per kg.$^{\frac{3}{4}}$ (p. 128). The results of two heifers have been calculated to an intake of 70 kg. calories metabolizable food energy per kg.$^{\frac{3}{4}}$. The method used is illustrated in table 9.

The results of these calculations are summarized in table 10. The partial efficiency thus derived from our results is high throughout compared with the efficiency for fattening which would be expected on the basis of the composition of the food. Using Kellner's figures (Kellner, '19, appendix, table 1) one may calculate for example that the 'Wertigkeit' (value) of ration 1 was 82.7%, that of ration 5, 86.5%. Kellner's further data (Kellner and Köhler, '00) for the metabolizable energy in digestible protein (4.96 kilocalories per gram) digestible fat (8.82 kilocalories per gram) digestible crude fiber (3.65 kilocalories per gram) and starch (3.76 kilocalories per gram) together with the partial efficiency of the metabolizable energy for fattening (namely, 45% for protein, 56% for fat, 63% for fiber and 59% for starch), lead to the calculation of a partial efficiency of 46.4% of the metabolizable energy for our ration 1 and 50.7% for ration 5.

The 'Wertigkeit' mentioned above has been used for this calculation.

If the net energy for these two rations is calculated according to Armsby's method (Armsby, '22, p. 674) a partial efficiency of 61% of the metabolizable energy results for ration 1 and 64% for ration 5. The fact that Armsby's values are higher than those of Kellner and our actual results still higher than the values obtained from Armsby's calculation is not surprising. Kellner's data are strictly fattening values for

TABLE 9

Calculation of partial efficiency at a food level of 70 kg. cal. metabolizable energy per kg.[1]

Animal 27

Heat production at an intake of 73.7 kg. cal. metabolizable food energy per kg.[1] (trial 13)	88.5 kg. cal. per kg.[1]
Heat increment per calorie metabolizable energy between an intake of 40.3 kg. cal. (trial 15) and 73.7 kg. cal. per kg.[1] (trial 13): 0.21 kg. cal.	
Heat increment for a difference of 3.7 kg. cal. metabolizable energy	0.8 kg. cal. per kg.[1]
Interpolated heat production at an intake of 70 kg. cal. per kg.[1]	87.7 kg. cal. per kg.[1]
Heat production during fasting (trial 17)	67.8 kg. cal. per kg.[1]
Calculated heat increment for increase of food energy from fasting to 70 kg. cal. per kg.[1]	19.9 kg. cal. per kg.[1]
Heat increment in per cent of increase in metabolizable food energy: $\frac{19.9}{70} \times 100 =$	28.5%
Partial energy efficiency between fasting and intake of 70 kg. cal. metabolizable food energy: $100 - 28.5 =$	71.5%

adult steers, Armsby's method is based on experiments at supermaintenance as well as submaintenance levels with steers ranging from 9 to 60 months of age (Armsby and Fries, '15, p. 438) and the partial efficiency in our trials was measured at submaintenance of young animals.

Kellner's as well as Armsby's method of calculating net energy leads to the conclusion that the partial efficiency of food B (particularly ration 5) should be higher than that of food A (particularly ration 1). Our data presented in table 10 are in agreement with this prediction.

During the preliminary period, when both heifers received dicalcium phosphate as a supplement to their phosphorus-deficient diet, the partial-energy efficiency was practically the same. During the second period of our trial, when animal 27 was changed to the phosphorus-low diet and developed symptoms of phosphorus deficiency, its partial-energy efficiency was decreased, while its mate that was still receiving dicalcium phosphate utilized the food energy with the same partial efficiency as in the preliminary period.

TABLE 10

Partial energy efficiency of phosphorus-deficient and control heifers. Calculated to a food level of 70 cal. metabolizable food energy per kg.[1]

	ANIMAL 27		ANIMAL 29	NO. 32		NO. 18
	Phosphorus deficient	Normal phosphorus intake	Normal phosphorus intake	Phosphorus deficient	Normal phosphorus intake	Normal phosphorus intake
	%	%	%	%	%	%
Food A rations (1 to 4)						
Preliminary period trials 2, 4 and 11		83.1	81.4		77.3	73.3
Second period of phosphorus deficiency trials 13, 15, 17	71.5		83.3	69.6		
Food B (rations 5 and 6)						
Third period of phosphorus deficiency trials 20, 22, 24	84.7		88.7			78.0

The partial efficiency of the phosphorus-deficient animal during the third period is again lower than that of its pair mate on the same diet supplemented with phosphate.

The partial efficiency of the Angus heifer, 32, at the start of the experiment was slightly below that of the two Hereford heifers. Phosphorus deficiency in this heifer also lowered the partial-energy efficiency, namely, from 77.3 to 69.6% during the second period of our experiment, thus confirming the observation with the Hereford heifer, 27. During the third period of the experiment heifer 32 had unfortunately lost its appetite to such a degree that it would not eat more than a one-half maintenance ration.

The decrease in partial-energy efficiency goes parallel with the decrease in the efficiency of protein utilization. The increase in the katabolism of protein in the phosphorus-deficient animals alone cannot, however, account for the entire increase in the calorigenic action of the phosphorus-deficient food.

5. SUMMARY

Two beef heifers fed a phosphorus-deficient diet containing only 0.13% of phosphorus ceased to grow after 6 months of the low-phosphorus regimen, maintained their body weight during the next year (with 0.09% phosphorus in the food), and finally when fed a diet still lower in phosphorus (with 0.068% phosphorus) lost weight. Two control heifers fed with the same food but supplemented with dicalcium phosphate so that the phosphorus content was above 0.4% increased their weight while their phosphorus-deficient pair mates ceased to grow.

The phosphorus-deficient animals developed bone-chewing and coprophagia. The inorganic phosphorus content in their blood dropped from 9.0 to 3.9 mg. of phosphorus per 100 cc. of blood serum while that of the controls remained at the initial level of about 9 mg. phosphorus per 100 cc. of serum.

Phosphorus deficiency had no effect on body temperature, digestibility and metabolizability of the food energy, R. Q. and fasting katabolism.

Phosphorus deficiency increased slightly the regression of CO_2 production and O_2 consumption on food intake (not proved with statistical significance). It decreased the partial efficiency of energy utilization, the efficiency of food protein for sparing body protein, and the appetite of the animals.

Phosphorus deficiency lowers the total efficiency of energy utilization ($\frac{\text{Energy in the product}}{\text{Total food energy}} = \text{mean partial efficiency} - \frac{\text{Total intake of food energy}}{\text{Fasting katabolism}}$) mainly by lowering the appetite and secondly by lowering the partial efficiency whereas it does not seem to influence the fasting katabolism.

ACKNOWLEDGMENT

The authors are indebted to G. H. Hart, who instigated the problem and was instrumental in organizing the extensive work necessary for its solution. The gas analyses necessary for the respiration trials were done by Helene K. Rohwer and Shizu Morey, and the rest of the analytical work by R. W. Caldwell and H. C. Johnson.

LITERATURE CITED

ARMSBY, H. P. 1922 The nutrition of farm animals. Macmillan Company, New York.

ARMSBY, H. P. AND J. A. FRIES 1915 Net energy values of feeding stuffs for cattle. J. Agr. Res., vol. 3, pp. 435–491.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS 1930 Official and tentative methods of analysis.

CLARK, E. P. AND J. B. COLLIP 1925 A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem., vol. 63, pp. 461–464.

ECKLES, C. H. AND T. W. GULLICKSON 1927 The relation of phosphorus deficiency to the utilization of feed. Am. Soc. An. Prod. Proc., pp. 16–21.

ECKLES, C. H., L. S. PALMER, T. W. GULLICKSON, C. P. FITCH, W. L. BOYD, L. BISHOP AND J. W. NELSON 1935 Effects of uncomplicated phosphorus deficiency on estrus cycle, reproduction and composition of tissues of mature dairy cows. Cornell Vet., vol. 25, pp. 22–43.

EZEKIEL, M. 1930 Methods of correlation analysis. 427 pp. John Wiley and Sons, New York.

FISHER, R. A. 1930 Statistical methods for research workers. 283 pp. Oliver and Boyd, Edinburgh.

FISKE, C. H. AND Y. SUBBAROW 1925 The colorimetric determination of phosphorus. J. Biol. Chem., vol. 66, pp. 375–400.

FORBES, E. B., W. W. BRAMAN, M. KRISS, C. D. JEFFRIES, R. W. SWIFT, R. B. FRENCH, R. C. MILLER AND C. V. SMYTHE 1928 The energy metabolism of cattle in relation to the plane of nutrition. J. Agr. Res., vol. 37, pp. 253–300.

GUILBERT, H. R. Normal variation in the inorganic phosphorus of the blood of beef cattle. Unpublished data.

HUFFMAN, C. F., C. W. DUNCAN, C. S. ROBINSON AND L. W. LAMB 1933 Phosphorus requirement of dairy cattle when alfalfa furnishes the principal source of protein. Michigan State College Tech. Bul. 134, pp. 1–75.

KELLNER, O. 1919 Die Ernährung der landwirtschaftlichen Nutztiere. 8 Aufl. 667 pp. Herausgegeben von G. Fingerling. Paul Parey, Berlin.

KELLNER, O. AND A. KÖHLER 1900 Untersuchungen über den Stoff- und Energiewechsel des erwachsenen Rindes bei Erhaltungs und Produktionsfutter. Landw. Vers. Sta. 53, pp. 1–474.

KLEIBER, M. 1932 Body size and metabolism. Hilgardia, vol. 6, pp. 315–353.

———— 1935 The California apparatus for respiration trials with large animals. Hilgardia, vol. 9, pp. 1–70.

KLEIBER, M., R. W. CALDWELL AND H. JOHNSON 1936 Losses of nitrogen and carbon in drying feces of cattle. Soc. Exp. Biol. and Med. Proc., vol. 34, pp. 128–130.

MCCRUDDEN, F. H. 1910 Quantitative separation of calcium and magnesium in the presence of phosphates and small amounts of iron devised especially for the analysis of foods, urine and feces. J. Biol. Chem., vol. 7, pp. 83–100.

———— 1911 The determination of calcium in the presence of magnesium and phosphates; the determination of calcium in urine. J. Biol. Chem., vol. 10, pp. 187–199.

MITCHELL, H. H., T. S. HAMILTON, F. J. MCCLURE, W. T. HAINES, JESSIE R. BEADLES AND H. P. MORRIS 1932 The effect of the amount of feed consumed by cattle on the utilization of its energy content. J. Agr. Res., vol. 45, pp. 163–191.

MOHLMAN, F. W. AND G. P. EDWARDS 1931 Determination of carbon in sewage and industrial wastes. Indus. and Eng. Chem. Anal. Ed., vol. 3, pp. 119–123.

NEAL, W. M. AND L. S. PALMER 1931 A method for the preparation and analysis of representative samples from the bovine skeletal structure. J. Agr. Res., vol. 42, pp. 107–113.

NEAL, W. M., L. S. PALMER, C. H. ECKLES AND T. W. GULLICKSON 1931 Effect of age and nutrition on the calcium phosphate/calcium carbonate ratio in the bones of cattle. J. Agr. Res., vol. 42, pp. 115–121.

PALMER, L. S., W. S. CUNNINGHAM AND C. H. ECKLES 1930 Normal variation in the inorganic phosphorus of the blood of dairy cattle. J. Dairy Sci., vol. 13, pp. 174–195.

RIDDELL, W. H., J. S. HUGHES AND J. B. FITCH 1934 The relation of phosphorus deficiency to the utilization of feed in dairy cattle. Kan. Agr. Exp. Sta. Tech. Bul. 36, pp. 1–54.

THEILER, A. 1933 Die Bedeutung des Kalziums und des Phosphors im Haushalte der Wiederkäuer. Schweiz. Landw. Monatsh. Repr., pp. 1–30.

VAN LANDINGHAM, A. H., H. O. HENDERSON AND G. A. BOWLING 1935 The effect of age and phosphorus intake on the calcium and inorganic phosphorus content of whole blood of dairy heifers. J. Dairy Sci., vol. 18, pp. 557–572.

WIEGNER, G. AND A. GHONEIM 1930 Über die Formulierung der Futterwirkung. Die Tierernährung, Bd. 2, S. 193–232.

THE ACTIVITY OF YEAST EXTRACT IN THE PREVENTION OF RENAL HYPERTROPHY CAUSED BY HIGH PROTEIN DIETS [1]

BERNARD B. LONGWELL, ROBERT P. JOHNSTON AND ROBERT M. HILL
Department of Biochemistry, University of Colorado School of Medicine, Denver

ONE FIGURE

(Received for publication April 28, 1936)

In a previous publication Longwell, Hill and Lewis ('32) reported that excessive amounts of protein in the diet of the white rat resulted in renal hypertrophy, an effect which could be prevented by the addition of sufficient amounts of yeast extract to the diet. These results were in agreement with those of Reader and Drummond ('26) and of Hartwell ('28), but in disagreement with the results of Francis, Smith and Moise ('31), a fact which was overlooked when the original paper was presented for publication. The evidence obtained by MacKay ('33) indicates that vitamin B (old terminology) is antagonistic to this action of casein. The earlier conclusions were drawn from a small part of a larger study concerned with the effect of cystine upon the kidney. In view of the disagreement between that study and the results of Francis, Smith and Moise, additional experiments concerning the effect of yeast extract on the enlargement of the kidney caused by casein are herein reported.

[1] Presented in part before the Division of Biological Chemistry at the eighty-sixth meeting of the American Chemical Society, September 11, 1933.

METHODS

The experimental procedure was the same as that formerly described (Longwell, Hill and Lewis, '32) with a few additions and modifications. Vitamin-free casein[2] was used as the protein. We formerly made use of alcohol-extracted casein prepared in our own laboratory. In the feeding regime of those animals which received a high protein diet, the casein was substituted for an equicaloric quantity of cornstarch in the Sherman and Spohn ('23) diet.

The undifferentiated yeast extract was made according to the method of Williams and Lewis ('30), and the amount fed daily is expressed in gram yeast equivalents (g.y.e.), the gram yeast equivalent being that amount of yeast extract obtained from 1 gm. of dry yeast. In the experiments in which the separated vitamins of the water-soluble B group were used, autoclaved liver supplied a source of vitamin G, and the vitamin B was fed in the form of tikitiki[3] extract (Evans and Lepkovsky, '30).

The experimental animals, male albino rats, were fed for a period of 21 days, beginning when they were 28 days old. They were killed by a head blow and the kidneys and hearts were weighed in glass-stoppered weighing bottles after the large blood vessels had been carefully removed from these organs. The kidney weights obtained are compared to the body weight and to the heart weight as a means of measuring renal hypertrophy. The measurements are expressed as the K:B (kidney:body weight) ratio and the H:K (heart:kidney) ratio, respectively. In our earlier study we used only the K:B ratio as a means of measuring renal hypertrophy. This method is possibly open to criticism because of the complicating factor of inanition to which the animals which received the restricted vitamin supplements were subject. Francis, Smith and Moise ('31) used the heart:kidney

[2] Obtained from The Casein Manufacturing Corporation of America.

[3] The yeast used in this investigation was furnished by Standard Brands, Inc., and the rice polishings were furnished by the Louisiana State Rice Milling Company. The cooperation of these companies is gratefully acknowledged.

ratio for the measurement of renal hypertrophy. This method rules out any possible effect of inanition provided the assumption is correct that there are no changes in the circulatory system (heart) as a result of the ingestion of abnormally great amounts of protein or abnormally small amounts of vitamin.

In a further attempt to rule out the factor of inanition, a comparison was made between the kidney weight and the fat-free body weight. It was thought that the fat-free body weight might be used in this manner because of the loss of body fat which occurs as the result of inanition from whatever cause. After removal of the kidneys and heart, the carcass of the animal was heated on a water bath in a strong solution of potassium hydroxide until it had dissolved completely. The solution was cooled, acidified with sulfuric acid, and extracted repeatedly in tall cylinders with petroleum ether. The extraction was accomplished by shaking the hydrolyzed carcass with ether and allowing the ether extract to separate, after which it was pipetted off and evaporated to a constant weight in a tared flask.

RESULTS

Tables 1 and 2 show the results of these experiments. They confirm our original contention that sufficient yeast extract as a dietary supplement inhibits the renal hypertrophy which occurs as a consequence of the ingestion of excessive amounts of casein. When the higher amounts of protein were fed (80% of the diet, table 2), the administration of large amounts of yeast extract did not completely prevent hypertrophy of the kidneys, but the larger quantities (3.0 and 4.0 g.y.e.) tended to inhibit this effect of casein.

Table 1 contains data on the relationship between the kidney weight and the fat-free body weight. The results show the same general trend when the extent of renal hypertrophy is measured in this manner as do the results in the same groups when the whole body weight is used as a basis of comparison. Figure 1 depicts the quantitative estimation of

the amount of fat present in the bodies of the rats of groups 1 to 10, inclusive.

Race, Longwell and Lewis ('32) made an attempt to show the effect of inanition on the K:B ratio. Food curtailment was used to retard growth so that the rats demonstrated

TABLE 1

The effect of yeast extract in the prevention of renal hypertrophy caused by casein

GROUP	NUMBER OF RATS	YEAST EXTRACT	CASEIN	K : B RATIO [2]	K : B RATIO (FAT-FREE BODY WEIGHT)	H : K RATIO [3]
		g.y.e.[1]	%			
1	16	1.0	18	0.012	0.012	0.408
2	6	0.0	40	0.019	0.020	0.288
3	6	0.2	40	0.014	0.014	0.343
4	5	0.4	40	0.013	0.014	0.359
5	6	0.6	40	0.013	0.014	0.361
6	6	0.8	40	0.013	0.014	0.375
7	6	1.0	40	0.013	0.014	0.393
8	6	1.5	40	0.012	0.013	0.373
9	2	1.5	80	0.021	0.021	0.326
10	3	2.0	80	0.018	0.019	0.333

[1] One g.y.e. (gram yeast equivalent) is the amount of extract which was obtained from 1 gm. of yeast.

[2] Ratio between the kidney weight and the body weight.

[3] Ratio between the heart weight and the kidney weight.

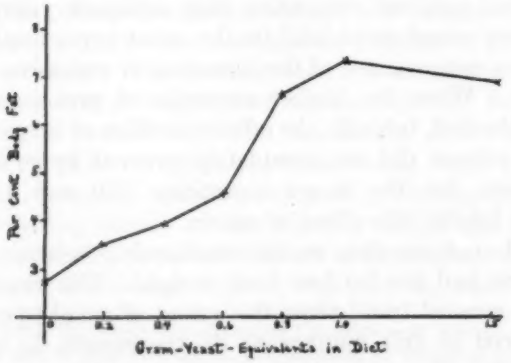


Fig. 1 Effect of yeast extract on body fat of rats on 40% protein diet.

approximately the same body weight change as a group deprived of yeast extract. The K: B ratio was 0.011 and indicates that inanition, per se, was not a factor which influenced the results herein reported.

Groups 27 and 28 (table 2) were fed autoclaved liver as a source of vitamin G and tikitiki extract as a source of vitamin B. The only supplement given to group 29 in addition

TABLE 2

The effect of yeast extract, autoclaved liver and tikitiki extract in the prevention of renal hypertrophy caused by casein

GROUP	NUMBER OF RATS	YEAST EXTRACT	CASEIN	K: B RATIO	H: K RATIO	AVERAGE WEIGHT GAIN
		g.y.e.	%			*gm.*
11	8	1.00	20	0.013	0.356	26.4
12	4	0.00	40	0.015	0.306	0.7
13	8	0.25	40	0.015	0.323	7.5
14	8	0.50	40	0.014	0.278	13.6
15	8	1.50	40	0.012	0.386	29.8
16	8	0.00	60	0.018	0.269	—2.9
17	8	0.25	60	0.017	0.295	0.5
18	8	0.50	60	0.017	0.215	8.4
19	8	1.50	60	0.017	0.318	31.7
20	8	3.00	60	0.014	0.341	34.8
21	4	0.00	80	0.027	0.248	—7.2
22	8	0.25	80	0.020	0.265	4.8
23	8	0.50	80	0.018	0.299	6.4
24	8	1.50	80	0.019	0.287	18.3
25	8	3.00	80	0.016	0.341	27.6
26	8	4.00	80	0.017	0.310	23.8
27	8	[1]	20	0.011	0.387	38.3
28	8	[1]	40	0.013	0.371	42.8
29	8	[2]	40	0.015	0.310	26.6
30	8	[3]	40	0.015	0.391	1.3

[1] One gram autoclaved liver and extract from 1 gm. of rice polishings daily.
[2] One gram autoclaved liver daily.
[3] Extract from 1 gm. of rice polishings daily.

to the basal diet was autoclaved liver. Group 30 received tikitiki extract as the only dietary supplement. The results with groups 27 and 28 show substantially the same relationship between the kidney weight and the other body measurements used as that established in the groups which received

yeast extract. There is no difference between the K:B ratio in group 29 (autoclaved liver) and group 30 (tikitiki). These data do not support the contention of Hartwell ('28) that the effective agent of yeast is the heat-stable factor, but are in agreement with the findings of Prunty and Roscoe ('33). We note this fact without undue emphasis because the results were obtained with two small groups only, and because liver and rice polishings were used as a source of the vitamins, a procedure which introduced substances into the dietary regime which were not identical with the factors obtained from yeast.

The data of group 30 (table 2) show that the animals which received tikitiki extract grew much more poorly than did those which received autoclaved liver (group 29). This result was probably due to the growth promoting properties of vitamin G. Furthermore, the growth of these animals was not greatly less than that of the animals which received whole yeast extract. According to Stiebeling and Alleman ('33) extraction with alcohol destroys part of the vitamin G. Since this vitamin is the limiting factor in the growth of the animals under the conditions of this experiment, we feel that the growth rates of the animals which received the undifferentiated vitamin B complex as compared to group 29 are due to partial destruction of vitamin G in the whole yeast extract. In spite of the differences in rates of growth, the results obtained on the weights of the kidneys appear to be significant.

DISCUSSION

The workers who have observed that yeast has an inhibitory effect upon renal hypertrophy caused by protein have usually attributed this effect to its vitamin B or G content. It will be recalled that Francis, Smith and Moise and Prunty and Roscoe have reported that whole yeast and the heat stable factor of yeast, respectively, do not inhibit renal hypertrophy. These latter findings are confirmed in this report. When one attempts to explain these negative results in the light of variations in the vitamin preparations, one encounters

the difficulty that no sharp separation has been made between the factors used. It is true that some of the results have been obtained with preparations which, according to the known properties of vitamins B and G, should contain one of these to the exclusion of the other. However, these preparations contain many additional vitamin components and until preparations of definite vitamin content have been studied or until the factors are used in the pure form, the relationship of accessory food substances to the development of the kidneys should not be attributed to any definite substance.

Reader and Drummond ('26) used whole yeast extract. The method of preparation is not described, but the material was active in the prevention of renal hypertrophy when large amounts were used. Hartwell ('28) found that untreated marmite gave positive results. MacKay ('33) utilized an Osborne and Wakeman ('19) concentrate prepared by alcoholic fractionation of a yeast extract made with very dilute acetic acid, and found it to be active. Our own results were obtained with an alcoholic extract of yeast. The methods of preparation of these various extracts are not identical, and the final products are probably quantitatively different with respect to vitamins B and G, and possibly they are qualitatively different with respect to the other factors present in yeast. These variations may be brought about by differences in the original yeast, and by destruction or by poor extraction of the dietary factors in the process of preparation. Even though these differences may exist, they do not seem to be significant, as far as the effectiveness of yeast is concerned, since all of the above mentioned workers obtained positive results with undifferentiated extracts.

Marmite which had been autoclaved 4½ to 5 hours was used effectively by Hartwell ('28) in part of her experiments. Opposed to this evidence stand the findings of Prunty and Roscoe ('33) who found autoclaved acetic acid extract of yeast inactive. Autoclaved liver was inactive in our own experiments. The use of this latter material, however, in all probability introduces factors into the diet which are not identical with those found in yeast. The existing evidence

does not permit allocation of the activity of yeast in the prevention of kidney hypertrophy to vitamin G.

Francis, Smith and Moise ('31) are the only workers who have reported a failure of yeast to counteract the effect of a diet rich in protein on the kidney. There is no evidence in their work that the diet did not contain the requisite accessory factors. The vitamin-containing supplements in question were given in the form of whole, dry yeast fed daily in amounts far in excess of that necessary to insure good growth. It is possible, however, that these factors were quantitatively insufficient to counteract the effects of such excessive levels of dietary protein (compare MacKay, '33). Additional supplements of autoclaved yeast and tikitiki extract were given to some of their animals without any effect, however, and this fact makes it likely that the yeast supply was adequate.

Critical analysis of these various reports forces one to the conclusion that differences in results must have been caused by some factors other than the accessory dietary supplements obtained from yeast. In our own work, groups 9 and 10, table 1, show only five animals. These represent the survivors of nineteen animals which were started on the feeding regime indicated. The only variable factor in these diets was the protein. Protein used at a level of 80% of the diet had a severe toxic effect on these animals. Not only did they fail to grow, but also they began to suffer from extreme inanition soon after the feeding was begun. In spite of the fact that they ate little and failed to consume double the amount of protein taken by the groups which received the 40% protein diet, fourteen of the nineteen rats died before termination of the experimental period of 21 days. No such evidence of intoxication was observed in the rats of groups 21 to 26, table 2 (second series). The diets of the animals of these latter groups were identical with those of groups 9 and 10 of the first series except that we had received a new supply of casein before their feeding was begun. It is possible that some difference in the preparation of the casein is an effective factor in these experiments. This hypothesis is being subjected to investigation.

A second possible explanation of these discrepancies appears upon examination of the data of Reader and Drummond ('26, p. 1261). It will be noted that in their groups C and D the kidneys of the animals of group C were larger than those of group D. The authors do not seem to consider that this difference is significant, but the kidneys of the animals of group C are 31.9% larger by reference to body weight than those of the animals of group D. The diets of these two groups differed only in that diet C contained no fat except that fed in the cod liver oil, whereas diet D contained 18 parts of 'hardened fat' in addition to the cod liver oil. It is of extreme interest that the only other experiments in which the diets contained no fat are those of Francis, Smith and Moise ('31), and their results show yeast to be ineffective as a preventative of renal hypertrophy. The possibility of fat as a key factor in the effectiveness of yeast in the prevention of this type of kidney enlargement is being investigated by us at the present time.

Our animals did not make quite normal growth. One might suppose that systemic growth may be a factor which influences the size of the kidneys. Reference to group 11, table 2, however, in which the protein of the diet was low, shows that there was no renal hypertrophy in spite of the fact that optimum growth was not attained. While one must admit that the growth element may be of influence, we feel that the difference in growth shown by the animals on the low and high protein diets was not sufficiently great to vitiate our conclusions.

SUMMARY

Young albino rats fed diets containing amounts of casein in excess of that necessary for optimum growth developed renal hypertrophy. This tendency was prevented or inhibited by yeast extract supplements. Neither tikitiki extract nor autoclaved liver prevented entirely the renal hypertrophy which resulted from a 40% protein diet, but they had an inhibitory effect when given together. The results were not due to inanition.

LITERATURE CITED

EVANS, H. M. AND S. LEPKOVSKY 1930 On the preparation of a concentrated source of the heat-labile vitamin B, free from contamination with the heat stable factor G. J. Nutrition, vol. 3, p. 353.

FRANCIS, L. D., A. H. SMITH AND T. S. MOISE 1931 Diet and tissue growth. VIII. Influence of vitamins B, G and undifferentiated B on the effects produced by protein-rich diets upon the kidney of the rat. Am. J. Physiol., vol. 97, p. 210.

HARTWELL, G. A. 1928 Protein and vitamin B. Biochem. J., vol. 22, p. 1212.

LONGWELL, B. B., R. M. HILL AND R. C. LEWIS 1932 The relation of the vitamin B complex to renal enlargement caused by cystine and protein in the diet of the rat. J. Nutrition, vol. 5, p. 539.

MACKAY, E. M. 1933 Factors which determine renal weight. XV. The relation between the vitamin B (old terminology) and protein intakes. Am. J. Physiol., vol. 106, p. 571.

OSBORNE, T. B. AND A. J. WAKEMAN 1919 Extraction and concentration of the water-soluble vitamine from brewers' yeast. J. Biol. Chem., vol. 40, p. 383.

PRUNTY, F. T. G. AND M. H. ROSCOE 1933 The vitamin B complex and high protein diets. Biochem. J., vol. 27, p. 699.

RACE, I. L., B. B. LONGWELL AND R. C. LEWIS 1932 The role of various fractions of the vitamin B complex in the prevention of the kidney enlargement produced by cystine in the diet. Unpublished data presented before the American Chemical Society, Denver, 1932.

READER, V. AND J. C. DRUMMOND 1926 Relation between vitamin B and protein in the diet of growing rats. Physiological role of vitamin B. Part II. Biochem. J., vol. 20, p. 1256.

SHERMAN, H. C. AND A. SPOHN 1923 A critical investigation and an application of the rat-growth method for the study of vitamin B. J. Am. Chem. Soc., vol., 45, p. 2719.

STIEBELING, H. H. AND I. L. ALLEMAN 1933 The relative extractability of vitamins B and G by plain and acidified alcohol. J. Am. Chem. Soc., vol. 55, p. 1477.

WILLIAMS, G. Z. AND R. C. LEWIS 1930 Evidence for the presence of a third factor in the vitamin B complex of yeast. J. Biol. Chem., vol. 89, p. 275.

A STUDY OF THE FECAL FLORA AND THE LINE TEST OF NORMAL RATS, RACHITIC RATS AND HEALING RACHITIC RATS [1]

HELEN FRIEDMAN

Department of Preventive Medicine, New York University College of Medicine

THREE FIGURES

(Received for publication March 26, 1936)

I. INTRODUCTION

A. Foreword

Although the line test is the official method for determining the extent of rickets or healing in the excised joint of an animal's leg, investigators (Jephcott and Bacharach, '26 and Grayzell and Miller, '28) have attempted to use the change in the pH of the feces as an indication of rickets or healing in the animal. This procedure when applied to children proved impracticable (Redman, '28).

Hess and Torrey ('32) tried to correlate the changes in the bacterial flora with the changes in pH by experimenting with the intestinal flora of rachitic rats and rats which were irradiated with ultraviolet rays.

B. Object of the experiment

The object of this investigation was to compare the pH and the bacterial flora of the feces of normal rats, rachitic rats and rats which were healed of rickets by feeding irradiated ergosterol and vitamin D milk.

[1] This paper was awarded a prize by the American Association of Medical Milk Commissions, Inc. and Certified Milk Producer's Association of America, Inc., under the auspices of the Milk Commission of the Medical Society of the County of New York.

II. EXPERIMENTAL

A. Feeding procedure

Twenty-eight- to 29-day-old albino rats weighing 48 to 50 gm. each were used. All rats had distilled water and Steenbock no. 2965 ration ad lib throughout the experimental period of 29 days. In addition to the basal diet, the following groups were fed special sources of vitamin D from the nineteenth through the twenty-ninth day (vitamin D testing period), the correct degree of rickets having been shown (line test) (Medical Research Council, '32) by the six test rats killed on the nineteenth day.

1. Negative controls
   a. Seventeen rats—nothing added to the basal diet.
   b. Milk controls
      (1) Five rats—6.0 cc. of plain certified milk fed to each rat.
      (2) Five rats—6.4 cc. of plain pasteurized A milk fed to each rat.
2. Healing rats
   a. Ten rats—irradiated ergosterol containing 2.7 U. S. P. X. vitamin D units was fed to each rat.
   b. Milk-fed rats
      (1) Five rats—6.0 cc. of vitamin D certified milk containing 2.7 U. S. P. X. units was fed each rat.
      (2) Five rats—6.4 cc. of vitamin D pasteurized A milk containing 2.7 U. S. P. X. vitamin D units was fed to each rat
3. Normal rats
   Eighteen rats used: Irradiated ergosterol containing 5.4 U. S. P. X. vitamin D units was fed to each rat every 4 days from the first day of the experiment throughout the 29 days.

B. Bacteriological methods

The routine bacteriological examination of the stools was carried out as recommended by Torrey ('26) and Hess and

Torrey ('32). Brom cresol purple lactose agar and blood agar plates were seeded with the stool suspensions (4 mm. loop = approximately 0.01 cc. was used) to determine the number of acid producing and non-acid producing organisms and green streptococci, hemolytic streptococci and coli respectively.

Plain beef agar plates were used to determine the bacterial count. All of the agar plates were read after 48 hours at 37°C.

The proteolysis and the quantity of gas produced were determined by inoculating cooked meat media covered with vaseline with the original suspension. Two tubes were inoculated with each fecal sample, one heated to 80°C. for 10 minutes to exclude all vegetative organisms. Both tubes were incubated for 4 days at 37°C. The proteolysis was measured by the amount of digestion taking place in the cooked meat media and the quantity of gas was measured by the height of the vaseline in the tube.

The pH of these fecal suspensions was determined by means of the Clark and Lubs color indicators (Clark, '25). The 1–10 dilution of fecal suspension was used with which to test the pH and this was compared with known standard buffer solutions.

From the brom creosol purple lactose plates, fishings were made and the organisms identified by subsequent cultures on milk medium, blood agar plates, lactose and dextrose broth.

C. Results

1. pH results. The range in pH of the feces of the normal rats was 6.20 to 6.60 throughout the entire 29-day period. Figure 1 shows the average pH of stool samples taken on the same day. The animals which gave these acid pH results showed a normal line test (no rickets) (Medical Research Council, '32).

The rachitic rats which were fed the Steenbock ration only had an acid pH (6.40 to 6.80) at the beginning of the test but as the rickets in the rat progressed, the pH became more basic (up to 7.60). The line test in these cases revealed

excellent rickets or moderate rickets. The average pH of these rats which became rachitic is shown clearly in figure 1. The rats fed the plain pasteurized and plain certified milk gave parallel results.

The pH of the feces of the healing rats on the other hand, was basic at the end of the nineteenth day but became more acid after the irradiated ergosterol containing vitamin D was fed. The average pH at the beginning of the healing period (nineteenth day) was 7.63 and at the end of the period

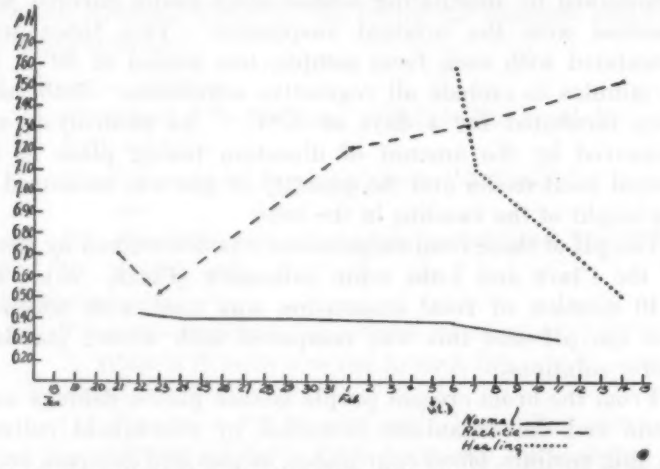


Fig. 1 Curves indicating the change in pH in the feces of normal, rachitic and healing rats. (Average pH.)

(twenty-ninth day) it was 6.53, almost hitting the normal curve. The line test checked these results with a 2 + healing The rats fed the vitamin D milk gave almost identical results.

2. Bacteriological results. Acid-producing colonies were predominant on the brom cresol purple lactose agar plates seeded with the fecal suspensions of the normal rats. This was apparent from every sample taken during the test period. As the rats became rachitic however, the number of acid-producing colonies grew smaller and the number of colonies producing no change in the indicator increased. The healing

rats showed a dominance of the latter colonies at the beginning of the vitamin D period but at the end of that period, whether the rats were fed the irradiated ergosterol or the vitamin D milk the plates showed an increase in acid-producing colonies almost consistently. The averages of these two types of colonies throughout the experiment are shown in figures 2 and 3.

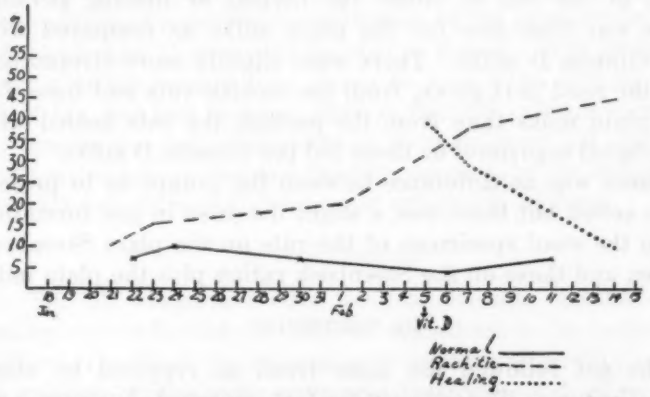


Fig. 2 Curves indicating the percentage of non-acid producing colonies from the feces of normal, rachitic and healing rats. (Average percentage.)

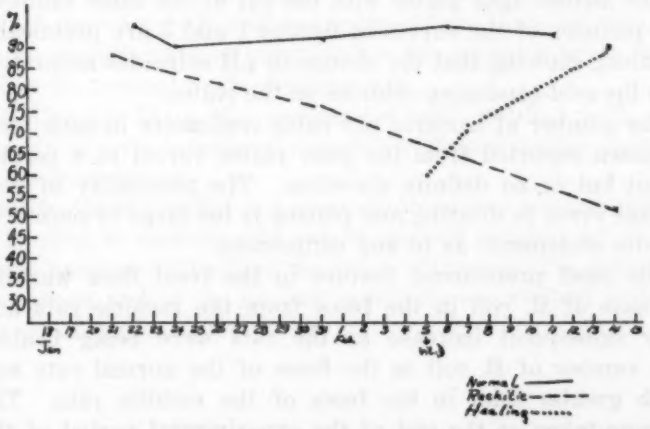


Fig. 3 Curves indicating the percentage of acid-producing colonies from the feces of normal, rachitic and healing rats. (Average percentage.)

The number of bacteria per cubic centimeter showed little difference between the feces of the various groups of rats tested.

The character of the bacterial flora recovered from the plates varied in the different groups. The abundance of the colon bacillus was observed to be definitely less on the plates from the samples taken at the end of the rachitic period than at the end of either the normal or healing periods. This was true also for the plain milks as compared with the vitamin D milks. There were slightly more streptococci for the most part green, from the rachitic rats and those fed the plain milks than from the normal, the rats healed with irradiated ergosterol or those fed the vitamin D milks.

There was no difference between the groups as to proteolytic action but there was a slight decrease in gas formation from the stool specimens of the rats on the plain Steenbock ration and those on the Steenbock ration plus the plain milk.

III. DISCUSSION

The pH followed the same trend as reported by other investigators. The interesting fact observed, however, was the almost direct correlation of the acid producing colonies on the lactose agar plates with the pH of the same samples. The pictures of the curves in figures 1 and 3 are practically identical, showing that the change in pH coincides accurately with the acid-producing colonies on the plates.

The number of bacteria per cubic centimeter in each fecal specimen reported from the pour plates varied to a certain extent but in no definite direction. The possibility of percentage error in diluting and plating is too large to permit of definite statements as to any differences.

The most pronounced feature in the fecal flora was the decrease of B. coli in the feces from the rachitic rats and their subsequent increase as the rats were being healed. The number of B. coli in the feces of the normal rats was much greater than in the feces of the rachitic rats. The average taken at the end of the experimental period of the percentage of B. coli showed the following:

Normal rats	15 % B. coli in the fecal flora
Rachitic rats	0.3% B. coli in the fecal flora
Healing rats	20 % B. coli in the fecal flora

There was also a slight increase in the green streptococci from the rachitic rat stools which was not apparent on the blood plates from the normal rats or the rats healed with irradiated ergosterol or vitamin D milk.

The pH according to the experimental results cited can be used roughly as a qualitative indication of the presence or the lack of vitamin D, provided the diet is known. It is doubted whether at the present time, it can be used alone without the aid of the line test because there is fluctuation from day to day among the individual rats as to their fecal pH during the experimental period and the time response of the different rats to the experimental diets is not sufficiently uniform.

IV. CONCLUSIONS

1. The pH reaction of the feces of normal, rachitic and healing rats indicated the type of organisms to be found in the feces, i.e., when the pH was acid (normal or healing rats) the colonies on the plate were acid producing and when the pH reaction was basic (rachitic rats) the colonies on the plate were non-acid forming.

2. A decrease of B. coli was evident from the stool samples of the rachitic rats as compared to the samples taken from either the normal or the healing rats.

3. Vitamin D in the pasteurized and certified milk and vitamin D in the irradiated ergosterol effected similar changes in the feces of rats.

4. Provided the diet is known, the tendency toward rickets or healing in rachitic rats can be gauged roughly by means of the basic or acid pH of the feces. However, the extent of the rickets and of the healing cannot be ascertained without the aid of the line test or x-ray.

ACKNOWLEDGMENTS

The author wishes to express her appreciation to Dr. K. George Falk and Miss Grace McGuire for their very excellent advice and encouragement and also to Miss Eugenia Valentine for her aid in planning this paper.

LITERATURE CITED

CLARK, W. M. 1925 The determination of hydrogen ions. Baltimore, Williams and Wilkins Company.

GRAYZELL, D. M. AND E. G. MILLER 1928 The pH of the contents of the gastro-intestinal tract of dogs, in relation to diet and rickets. J. Biol. Chem., vol. 76, p. 423.

HESS, A. AND J. C. TORREY 1932 The intestinal flora of rachitic rats before and after treatment with ultra-violet rays. Proc. Soc. Exp. Biol. and Med., vol. 29, pp. 761–766.

JEPHCOTT, H. AND A. L. BACHARACH 1926 A rapid and reliable test for vitamin D. Biochem. J., vol. 20, p. 351.

MEDICAL RESEARCH COUNCIL 1932 Vitamins: A survey of present knowledge. London, p. 84.

REDMAN, THERZA 1928 The hydrogen-ion concentration of the feces of rachitic children. Biochem J., vol. 22, p. 14.

TORREY, J. 1926 A comparatively simple technic for the bacteriologic study of fecal material. J. Infect. Dis., vol. 39, pp. 351–369.

STUDIES ON THE BLOOD AND TISSUES IN NUTRITIONAL MUSCULAR DYSTROPHY

S. MORGULIS AND HOWARD C. SPENCER

Department of Biochemistry, University of Nebraska College of Medicine, Omaha

TWO FIGURES

(Received for publication May 16, 1936

STUDIES ON THE BLOOD

Muscular dystrophy was experimentally produced in rabbits by means of the diet 13 of Goettsch and Pappenheimer ('31). As we reported elsewhere (Morgulis and Spencer, '36 a) recovery from dystrophy which in the course of time leads to complete regeneration of the diseased muscles can be effected by the addition of proper foodstuffs to the basic diet. Using both dystrophic rabbits and those recovering from this disability, we made a systematic study of the blood in the hope that these findings would shed some light on the problem of the causation of this disease.

METHODS

Blood was obtained from the marginal ear vein, xylol being used when necessary to cause local hyperemia. Sugar determinations were made on blood filtrates prepared according to the Somogyi procedure ('30) and the titrations carried out by the Shaffer-Somogyi method ('33). For the sugar tolerance tests the usual dosage of 3 gm. glucose per kilogram of body weight were administered by stomach tube to rabbits fasting 24 hours. Lactic acid was determined by Wendel's method ('33). The phosphorus determinations were all made by the Kuttner method (Kuttner and Cohen, '27; Kuttner

and Lichtenstein, '30) following the system of analyses outlined by Kuttner and Lichtenstein ('32). For the determination of the acid soluble-P 2 cc. of freshly drawn whole blood were immediately mixed with 18 cc. of 7% trichloracetic acid to precipitate the proteins. The filtrate represented a 1:10 dilution of the original blood. The adenosinetriphosphate (ATP) content was calculated by multiplying the difference between the P after hydrolysing for 7 minutes at 100° with N H_2SO_4 and the inorganic-P of the filtrate by 3/2. The lipid-P and cholesterol were determined in the alcohol-ether extract of the blood prepared according to Bloor ('18). The cholesterol was determined colorimetrically by the acetic anhydride reaction of Liebermann-Burchard.

THE GLYCEMIC CURVE

For several years, studying clinical cases of myopathy, we noted in patients with myotonic dystrophy a peculiar and characteristic zig-zag glycemic curve. The sugar tests were carried out by the usual routine following the administration of a standard quantity of glucose to the patients. The shape of the curve, which may be interpreted as manifesting an endocrine disbalance, is not accidental and repeats itself in the same individual with remarkable consistency. We had an opportunity to perform five tests on the same person over a period of 9 months, both before and after treatment with cortin, and except for minor deviations the different curves could be superimposed upon each other. In general, after glucose administration, the glycemic level does not rise as high as in normal individuals, and in some patients the rise is quite insignificant. The peak of the curve is reached in from 30 to 60 minutes, and is followed by a sharp sudden drop, sometimes 15 to 35 mg.% below the fasting level, to rise once more, though less steeply than previously. The curve then slopes off again. In a few instances, where the urinary sugar excretion was also determined, a marked glycosuria (up to 450 mg. glucose) was found at the end of 60 minutes.

In view of this experience with clinical cases of muscular dystrophy we deemed it worth while to study the sugar curve in rabbits suffering from nutritional muscular dystrophy. The average blood sugar value in eight control rabbits was found to be 81 mg.% (63 to 105 mg.%) and in eighteen dystrophic rabbits 78 mg.% (57 to 119 mg.%), showing practically no essential difference between the two groups of

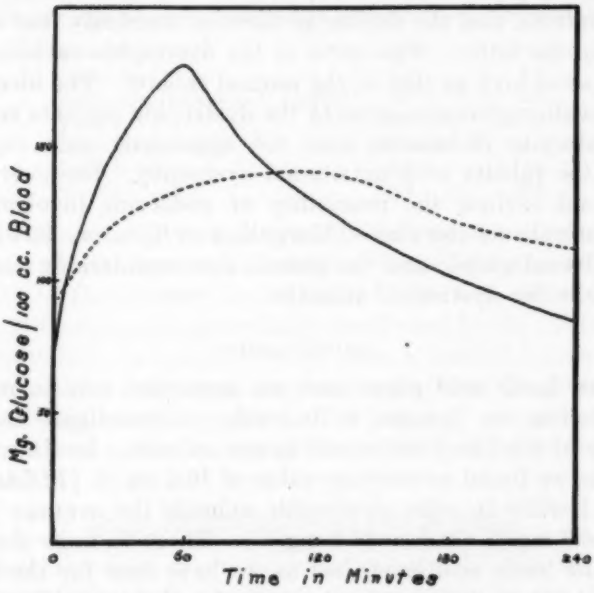


Fig. 1 Glycemic curve of normal rabbits (continuous line) and of dystrophic rabbits (broken line). These are composite curves.

animals. In figure 1 we reproduce the sugar tolerance curves obtained with normal and dystrophic rabbits. These are not individual but composite curves representing the general course of the glycemic reaction. It is obvious that the glycemic curve obtained with rabbits afflicted with muscular dystrophy of nutritional origin is entirely different from the curve prevailing among the human patients with myotonic

dystrophy. Of course, this points to the fact that these two conditions are probably totally different entities, although the histological study of the affected muscles in both instances indicates a remarkable degree of similarity in their microscopic appearance.

The glycemic curve of the dystrophic rabbits varies, however, from the normal curve in that its rise is less steep and slower, the peak being reached in 2 instead of in 1 hour, as in the controls, and the decline is likewise decidedly less sharp than in the latter. The curve of the dystrophic rabbits does not rise as high as that of the normal rabbits. The idea that the peculiar glycemic curve of the dystrophic patients reflects an endocrine disbalance does not apparently gain support from the rabbits with nutritional dystrophy. However, this does not exclude the possibility of endocrine involvement. We have shown elsewhere (Morgulis and Spencer, '36 b) that the adrenal glands and the gonads are considerably reduced in size in the dystrophic animals.

LACTIC ACID

Since lactic acid plays such an important role in muscle metabolism we thought it desirable to investigate the behavior of the blood lactic acid in our animals. In six control rabbits we found an average value of 16.6 mg.% (12.6 to 22.4 mg.%) while in eight dystrophic animals the average value was 16.3 mg.% (11.9 to 20.0 mg.%). These findings showing that the lactic acid level, just as we have seen for the blood sugar level, is the same in the control and dystrophic rabbits lead to the conclusion that neither of these constituents is associated with the factors responsible for the production of dystrophy.

ACID SOLUBLE PHOSPHORUS COMPOUNDS

Continuing the investigation of substances which play an active part in muscle metabolism, we determined quantitatively the various phosphorus fractions in the blood of our rabbits. The results of these determinations (on five normal and fourteen dystrophic rabbits) are tabulated below.

Since the acid soluble phosphorus compounds are found only in the red blood cells, the analytical results for the various fractions determined have been recalculated on the basis of the red cell count and corrected for 5.7 million cells per cubic millimeter, which was found to be the average for the control rabbits. A comparison, on this basis, shows that the distribution of the acid-soluble-P fractions is practically identical in the bloods from both normal and dystrophic rabbits, as can be seen from this summary of the results:

Phosphorus fraction	*Normal*	*Dystrophic*
Inorganic-P	5.2	5.9
Adenosinetriphosphate-P (ATP)	7.2	8.0
Ester-P	36.5	36.1
Total acid-soluble-P	40.7	41.8
Lipid-P	9.2	12.3
Total-P	52.6	60.2

It can be seen from these results that the total blood P is increased in the dystrophic rabbits, this being apparently due largely to the increase in the lipid-P fraction, but that the other P fractions are practically unaffected by the dystrophy. Not only does the lipid-P content show a decided increase but the cholesterol is likewise very much greater in the dystrophic than in the control rabbits. The marked changes in the alcohol-ether extractives are considered in detail in the next section.

LIPID PHOSPHORUS AND CHOLESTEROL

Since our study of the blood from animals with muscular dystrophy disclosed significant changes only in the lipid-P and cholesterol, we subjected these blood constituents to a more extensive investigation.

A study made on fourteen normal rabbits shows that the average for cholesterol is 115 mg.% and that for lipid-P 9.2 mg.%. In contrast to these normal values, analyses made on nineteen dystrophic rabbits give an average of 237 mg.% for cholesterol and 13.5 mg.% for the lipid-P content (table 1). This represents an increase in the cholesterol concentration of 107% and in the lipid-P of 45%.

The increase in the cholesterol of the blood apparently runs parallel to the degree of dystrophy, and while the lipid-P likewise seems to bear a relationship to the extent of the dystrophic change, this is not nearly as definite as in the case of the cholesterol. Using the number of plus signs as an arbitrary scale for expressing the degree of dystrophic change in the skeletal muscles, as determined by the microscopic examination of biopsy material, this relationship is

TABLE 1

Blood cholesterol and lipid-P (milligrams per cent) in dystrophic rabbits

RABBIT	FROM CRITICAL POINT [2]		CHOLESTEROL	LIPID-P
	Number of days	Loss of weight in grams		
254	3	90	293	12.5
256	9	400	292	14.7
258	4	140	279	15.4
260 (+++)[1]	12	330	260	12.5
264	4	130	242	12.8
266	3	230	222	10.8
274 (+)	6	270	205	12.4
275 (++)	12	420	233	13.0
279	14	420		13.8
280	6	140	213	12.8
282	4	70	216	12.6
286 (++++)	3	230	293	14.3
296	5	70	242	12.5
303	6	80	184	14.0
306	4	280	235	
313	7	390	286	15.6
319	6	180	250	
328	4	30	240	16.6
329	6	50	316	
Average			250	13.5

[1] The number of plus signs serves as a scale for expressing the degree of muscular degeneration judged on the basis of histological examination.

[2] The 'critical point' is a point on the growth curve of the dystrophic rabbit at which the body weight decreases sharply. For several days preceding this, the growth of the animal instead of being continuous begins to waver, losing a little one day and gaining the next. Definite metabolic changes occur at this period which will be discussed in a separate paper. When the sharp and large loss of weight takes place at the 'critical point' death of the animal is imminent unless recovery is brought about by special dietary means.

shown in the case of a few rabbits for which both the chemical and the histological data are available.

Rabbit no.	*Biopsy findings*	*Cholesterol, mg.%*	*Lipid-P, mg.%*
274	+	205	12.4
275	++	233	13.0
260	+++	260	12.5
286	++++	293	14.3

This view that the changes in the blood lipid-P and especially in the cholesterol are proportional to the development of dystrophy in the muscle system is borne out by

TABLE 2

Blood cholesterol and lipid-P (milligrams per cent) in rabbits which are recovering from definite dystrophy

RABBIT	ON RECOVERY DIET		CHOLESTEROL	LIPID-P
	Number of days	Gain of weight in grams		
280	91	1070	208	12.4
288	17	300	171	13.0
291	63	1060	182	9.8
294	95	1770	235	10.4
297	104	1220	125	7.7
298	100	910	118	9.3
300	15	90	261	14.1
300	97	1550	143	9.1
303	15	350	133	10.9
303	59	1140	250	12.4
328	9	200	204	13.6
328	13	290	129	11.0
328	60	1050	100	
Average			174	11.1

further studies. In six rabbits approaching dystrophy the average values for cholesterol and lipid-P were 215 mg.% and 10.4 mg.%, respectively. These values are considerably higher than those found in the control animals but less than those in rabbits with manifest dystrophy (table 1). The results obtained with rabbits recovering from dystrophy on our special diets are even more striking, as can be seen from the data in table 2. With the progress of recovery both the cholesterol and the lipid-P of the blood tend to decrease, the

average values in this group, in spite of its heterogeneous make-up, falling between those of the normal and of the dystrophic groups.

In this connection the data reported in table 3 are particularly significant because they were obtained on the same rabbits in the course of development of the dystrophy and bear out in a striking manner the direct relationship between the gradual rise in the cholesterol and in the lipid-P of the blood as the myopathy progresses. We also followed the changes

TABLE 3

Blood cholesterol and lipid-P (milligrams per cent) in rabbits during the progressive development of dystrophy

RABBIT	DAYS ON DIET	CHANGE IN WEIGHT IN GRAMS (AFTER CRITICAL POINT)	CONDITION	CHOLESTEROL	LIPID-P
306	48	— 30	Slightly dystrophic	172	
306	52	+ 40	Slightly dystrophic	161	
306	56	— 200	Dystrophic	178	
306	57	— 280	Severely dystrophic	235	
266	51	— 230	Dystrophic	222	10.8
266	57	— 430	Severely dystrophic	283	11.8

during recovery from the dystrophy. In one rabbit with dystrophy, whose blood cholesterol and lipid-P were 240 mg.% and 16.6 mg.%, respectively, we brought about recovery from the disease. We determined the cholesterol and lipid-P of the blood on the ninth, thirteenth and sixtieth day, when the animal had gained 200, 290 and 1050 gm. in weight, and found that the cholesterol changed to 204, 129 and finally 100 mg.% while the lipid-P decreased to 13.6 and 11.0 mg.%.

STUDIES ON TISSUES

In the preceding section we discussed the changes in the blood of rabbits suffering from nutritional muscular dystrophy. Here we shall consider the chemical changes in the tissues, more particularly in the muscles.

METHODS

Removal of muscle. The rabbits were anesthetized with sodium ortal (100 mg. per kilogram body weight by stomach tube), though toward the end of these experiments chloretone (200 mg. per kilogram) was substituted as the anesthetic, because this proved much more satisfactory in securing complete relaxation over a long period. The gastrocnemius muscle was very carefully dissected from the surrounding structures with as little trauma as possible and was frozen in situ by a mixture of solid carbon dioxide with ether or ethyl chloride, according to the technic described by Davenport and Davenport ('28). The muscle was frozen almost instantly without any noticeable contraction.

For the determination of the glycogen in muscle we used the Good, Kramer and Somogyi ('33) modification of the well-known Pflüger procedure and analyzed the sugar in aliquots, after preliminary hydrolysis, by the Shaffer-Somogyi method ('33). The glycogen content of the muscle was expressed as milligrams glucose per 100 gm. fresh tissue.

For the determination of the total acid soluble-P as well as its various fractions 1 to 2 gm. of the sliced frozen muscle were quickly extracted with ice cold 5% trichloracetic acid, using 10 cc. per gram of tissue. All the phosphorus determinations were carried out according to the Kuttner method (Kuttner and Cohen, '27; Kuttner and Lichtenstein, '30, '32). The inorganic-P was determined in the original trichloracetic acid filtrate, while the room temperature hydrolysis was made on the filtrate diluted 1 to 10. The pyrophosphate fraction was determined as in the case of the blood. The hexosemonophosphate-P was determined by the procedure of Cori and Cori ('31–'32).

The total creatinine determination was usually made on muscle which was frozen in situ. The early analyses were made by the method described by Rose, Helmer and Chanutin ('27) which requires about a gram of tissue. Subsequently we followed the micro-procedure of Ochoa and Valdecasas ('29) using about 100 mg. of tissue instead. We also found

that the trichloracetic acid filtrate prepared for the P determinations could be used satisfactorily in the Ochoa creatinine determination, developing the color with 5% NaOH and matching against a 0.1 mg. standard.

For the lipid-P and the cholesterol determinations the finely ground tissue was extracted with the alcohol-ether mixture. For this the tissue did not need to be frozen in situ. Generally we used 50 cc. of the Bloor mixture to extract a gram of tissue, repeating the extraction three times. The analysis of the lipid-P and the cholesterol was carried out in the same manner as in the case of the blood extracts.

MUSCLE GLYCOGEN

Since glycogen plays such an important role in muscle metabolism we turned our attention first of all to this substance. In table 4 the results of the determinations of muscle glycogen are reported. The average glycogen content in normal rabbits (after 1 day of fasting) was found to be 556 mg.% whereas the average for eighteen dystrophic animals was only 119 mg.%.

This enormous loss of glycogen in the dystrophic muscles is not a starvation effect. In a normal rabbit fasting 3, 6 and even 14 days we still found 358, 273 and 149 mg.% glycogen, respectively. Although the diseased animals do partake sparingly of food, none of the dystrophic rabbits naturally undergo anything approaching such a degree of inanition. The data in table 4 have been arranged in the order of a diminishing glycogen content of the muscles and comparing these with the findings of the histological examination of the muscles it is seen that a close relationship exists between the degree of degeneration and the loss of glycogen. However, neither the relative nor the absolute loss in weight, nor the length of time during which weight was lost, indicates the degree of dystrophy or the extent of the change in glycogen content. Further evidence for the correlation between the glycogen content and the extent of the muscular degeneration is found in the study of the two rabbits (213 and 280) which

have recovered from the attack of dystrophy and in which a muscle glycogen content of 308 and 273 mg.%, respectively, was observed.

TABLE 4

Muscle glycogen in normal and dystrophic rabbits (milligrams per 100 gm. fresh tissue)

RABBIT	LOSS OF WEIGHT IN GRAMS	PER CENT LOSS OF WEIGHT	DAYS ON DIET	GLYCOGEN (AS GLUCOSE)	CONDITION OF RABBIT
223	0	0		716	Normal (fasted 1 day)
307	70	3		596	Normal (fasted 1 day)
215	60	2		476	Normal (fasted 1 day)
281	100	6		434	Normal (fasted 1 day)
303	170	5		358	Normal (fasted 3 days)
253	240	9		273	Normal (fasted 6 days)
206	750	32		149	Normal (fasted 14 days)
213	100	5		308	Recovered
280	20	1		232	Recovered
292	240	22	33	218	Dystrophic (diet 13 + magnesium gluconate)
254	190	16	32	209	Dystrophic
226	250	30	31	187	Dystrophic
296	80	5	54	150	Dystrophic (+)
208	80	3	113	143	Dystrophic (++)
306	270	13	72	141	Dystrophic
256	400	31	33	139	Dystrophic
279	420	28	54	139	Dystrophic
285	450	29	43	126	Dystrophic (++++)
227	250	28	37	113	Dystrophic
286	230	17	53	109	Dystrophic (++++)
209	45	7	28	107	Dystrophic
258	220	17	30	93	Dystrophic
266	530	29	58	92	Dystrophic
313	390	31	37	83	Dystrophic
288	530	26	66	81	Dystrophic (++++)
263	260	20	47	81	Dystrophic (+++)
260	460	20	40	74	Dystrophic (+++)
275	400	25	53	69	Dystrophic

These studies suggest a possible relationship between the carbohydrate metabolism and the development of the muscular dystrophy. The question has naturally arisen as to whether it would be practicable to prevent or at least to alleviate the disease by maintaining the muscle glycogen depot

intact. Franke ('34) reported that the muscle and liver glycogen content can be materially increased by the administration of magnesium gluconate. We performed a number of experiments feeding rabbits this substance together with the dystrophic diet 13 but this did not affect either the severity or the onset of muscular dystrophy in the test animals. In rabbit 292, belonging to this series, we found a fairly high glycogen content (218 mg.%) at the time when the dystrophy had developed. Obviously, the loss of muscle glycogen is not the cause but rather a symptom of the dystrophic reaction.

ACID SOLUBLE PHOSPHORUS COMPOUNDS

The acid soluble phosphorus compounds constituting such an essential link in the chemical mechanism of muscular activity, we anticipated that an investigation of the changes occurring at the onset of dystrophy would yield significant data for an interpretation of the nature of the dystrophic process. To facilitate the survey of these results we present graphically in figure 2 the absolute as well as the relative distribution of the phosphate compounds in normal and in dystrophic rabbit muscles and for purposes of comparison we also give the analytical data gleaned from the literature. The results we found for the normal rabbit muscle are in good general agreement with those reported by other investigators. The results obtained with the muscle from dystrophic animals show considerable individual variation, as might be expected. However, the total acid soluble-P is noticeably reduced in most cases analyzed, and the decrease generally is proportional to the extent of the degeneration determined histologically. But though the absolute quantity of the acid soluble-P and of its various fractions is decreased, the relative distribution of the different P fractions is hardly at all affected. The per cent of phosphagen in both normal and dystrophic muscles is the same, the inorganic phosphate and the undetermined acid soluble-P fraction is somewhat greater and the hexosemonophosphate is smaller in the degenerated muscles. However, even these small changes still fall within

the range of normal variations. In this respect our results on the rabbit muscles are in good agreement with the results obtained by Nevin ('34) on biopsy material from human myopathy cases.

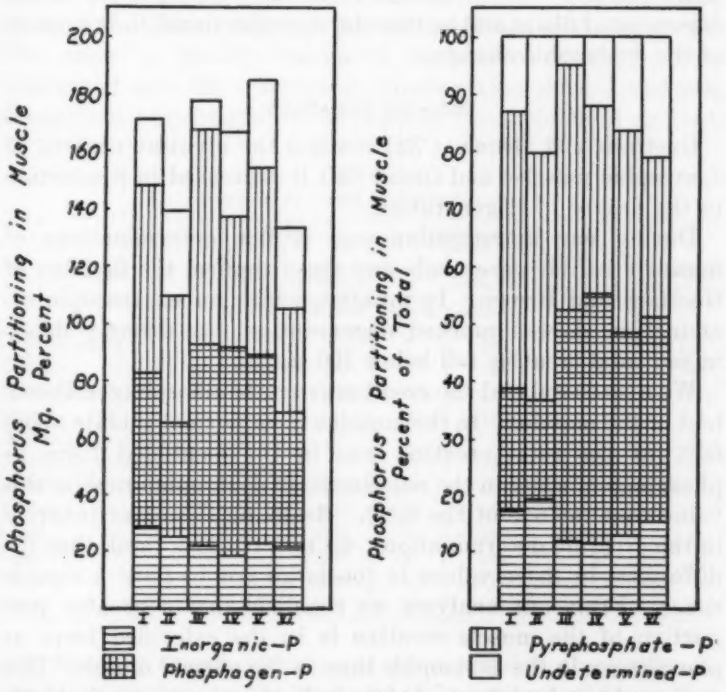


Fig. 2 The absolute and relative partitioning of the acid soluble phosphorus compounds of muscles from normal and dystrophic rabbits. Columns I, II, III and IV are based on the results of analyses of normal rabbit muscles obtained from the literature (Kerr and Blish, '32; Milroy, '31; Nevin, '34; Sacks and Sacks, '33). Columns V and VI are based on our own analyses of normal and dystrophic rabbit muscles, respectively.

The fact that the relative distribution of the various P fractions in muscles from normal and from dystrophic rabbits is essentially similar fits in well with the histological picture of the muscles. Even in muscles affected by extensive degeneration normal fibers are found amidst the debris of de-

generated fibers. The acid soluble-P compounds being intracellular components, the normal relative distribution would indicate that in the intact muscle fibers the chemical structure is likewise maintained intact, but the decrease in the total acid soluble-P content results from the disintegration of the degenerated fibers and is, therefore, proportional to the extent of the dystrophic change.

MUSCLE CREATINE

Goettsch and Brown ('32) studied the creatine content of dystrophic muscles and found that it decreased in proportion to the degree of degeneration.

During our investigation only a few determinations of muscle creatine were made and these confirm the findings of Goettsch and Brown. In muscles, which on microscopic examination showed marked degeneration, the directly determined total creatine fell below 100 mg.%.

We also calculated the creatine from the phosphagen-P content of the muscles. In the muscles from normal rabbits about 60% of the total creatine was in the combined form as phosphagen, while in the muscles from dystrophic rabbits this value rose to 76% of the total. Realizing the error inherent in the creatine determinations, we nevertheless think that the difference in these values is too large not to have a significance. From our analyses we conclude that a greater proportion of the muscle creatine is in the esterified form as phosphagen in the dystrophic than in the normal muscle. This may perhaps be interpreted to indicate a compensatory adjustment in the chemical structure of the muscle.

CHOLESTEROL

In view of the pronounced hypercholesteremia which we observed in the dystrophic rabbits it seemed advisable to investigate the cholesterol content of the muscles. Later, when we discovered that the cholesterol of the degenerating muscles is two and three times as great as that of the normal muscles, we extended our investigation to include not only the

gastrocnemius muscle but several other skeletal muscles as well as smooth muscles, the heart, and the various visceral organs and brain. The results of this study are summarized in table 5.

All the skeletal muscles examined, the gastrocnemius, gluteus and the muscles of the foreleg from dystrophic rabbits show a greatly increased cholesterol content. The abdominal and the intercostal muscles also show a definite, though not as pronounced, increase in the cholesterol content.

TABLE 5

Summary of tissue cholesterol studies

TISSUE	NORMAL		DYSTROPHIC		DIFFERENCE IN MILLIGRAMS PER CENT	PER CENT CHANGE
	Number of determinations	Average milligrams per cent cholesterol	Number of determinations	Average milligrams per cent cholesterol		
Gastrocnemius	6	65	7	189	+ 124	+ 191
Gluteus	6	54	4	99	+ 45	+ 83
Foreleg muscle	5	67	4	115	+ 48	+ 72
Heart	4	124	2	124	0	0
Abdominal muscles	2	53	2	78	+ 25	+ 47
Intercostal muscles	2	79	2	104	+ 25	+ 32
Intestines and stomach	4	165	2	170	+ 5	+ 3
Kidney	4	320	2	332	+ 12	+ 4
Lung	5	502	3	432	— 70	— 14
Liver	5	320	4	294	— 26	— 8
Brain	5	1866	4	1914	+ 48	+ 2
Spleen	3	414	3	329	— 85	— 20

The heart, stomach, kidney and brain exhibit practically no variation so far as the cholesterol is concerned from that found in the normal rabbits. This is especially true for the heart. In the case of the liver, lung and spleen the cholesterol content in the dystrophic animals is less than in the normal ones, the relative decrease being smallest in the liver and largest in the spleen.

This very striking and enormous increase in the cholesterol content of the skeletal muscles from dystrophic rabbits, coupled with the observation that the cholesterol content of other muscular structures (heart, smooth muscle) and of

several organs remains practically unchanged, suggests a severe disturbance in the cholesterol metabolism originating primarily in the muscle tissue itself. The limitation of the significant cholesterol changes to skeletal muscle places nutritional dystrophy more definitely among the myopathic disorders.

The fact that the heart from dystrophic rabbits has a normal cholesterol content fits in well with the findings of Goettsch and Pappenheimer ('31) that the cardiac muscle in such animals is also normal from the morphological standpoint.

SUMMARY

1. In rabbits suffering from nutritional muscular dystrophy we do not find the characteristic zig-zag sugar tolerance curve generally occurring in human subjects with muscular dystrophy. The glycemic curve of the dystrophic rabbit differs, however, from that of the normal rabbit in that it rises slower, reaching a peak in about twice the length of time, does not attain such a high level and slopes off more gradually than in the latter.

2. The analyses of a number of fasting bloods from both normal and dystrophic rabbits fail to indicate any appreciable variation between the two groups so far as the sugar, lactic acid, total acid soluble phosphorus and the partition of its several fractions are concerned.

3. The lipid-P and cholesterol content of the blood show marked increases in the diseased animals, the increase being proportional to the degree of the dystrophic change. When by proper dietary means the regenerative process in the dystrophic muscle is initiated leading to recovery from the disease the concentration of the lipid-P and particularly of the cholesterol in the blood decreases again, returning ultimately to the normal values.

4. The glycogen content of the skeletal muscles from rabbits suffering from nutritional muscular dystrophy is very greatly reduced, the reduction being proportional to the extent of the degenerative process.

5. The absolute concentration of the acid soluble phosphorus and its different fractions is decreased in the dystrophic muscles, the decrease being proportional to the degree of dystrophy, but the relative distribution of the various phosphorus fractions is practically unaltered and is nearly the same as in the normal muscles.

6. The creatine content of the muscles diminishes parallel to the development of dystrophy but the portion of the total creatine esterified in the form of phosphagen is considerably greater in the dystrophic than in the normal muscles.

7. The cholesterol content of the skeletal muscles is greatly increased in dystrophic rabbits. In the muscles of the extremities the cholesterol content may become even more than doubled while in the muscles of the trunk it is not so large. The cholesterol content of the muscles from visceral organs (heart, stomach, intestine) is practically unchanged, which is true also for the brain and kidneys, but in liver, lung and spleen there is a variable but definite decrease.

LITERATURE CITED

BLOOR, W. R. 1918 Methods for the determination of phosphoric acid in small amounts of blood. J. Biol. Chem., vol. 36, p. 33.

CORI, G. T. AND C. F. CORI 1931–1932 A method for the determination of hexosemonophosphate in muscle. J. Biol. Chem., vol. 94, p. 561.

DAVENPORT, H. A. AND HELEN K. DAVENPORT 1928 The latic acid content of resting mammalian muscle. J. Biol. Chem., vol. 76, p. 651.

FRANKE, HANS 1934 Magnesium und Kohlehydratstoffwechsel. Arch. Expt. Pathol. Pharmakol., vol. 174, p. 727.

GOETTSCH, MARIANNE AND ELIZABETH F. BROWN 1932 Muscle creatine in nutritional muscular dystrophy of the rabbit. J. Biol. Chem., vol. 97, p. 549.

GOETTSCH, MARIANNE AND ALWIN M. PAPPENHEIMER 1931 Nutritional muscular dystrophy in the guinea pig and rabbit. J. Exp. Med., vol. 54, p. 145.

GOOD, C. A., H. KRAMER AND MICHAEL SOMOGYI 1933 The determination of glycogen. J. Biol. Chem., vol. 100, p. 485.

KERR, STANLEY E. AND M. ELEANOR BLISH 1932 The effect of insulin on the phosphorus compounds of muscle. J. Biol. Chem., vol. 97, p. 11.

KUTTNER, THEODORE AND HARRIET R. COHEN 1927 Micro colorimetric studies. I. A molybdic acid, stannous chloride reagent. The micro estimation of phosphate and calcium in pus, plasma and spinal fluid. J. Biol. Chem., vol. 75, p. 517.

KUTTNER, THEODORE AND LOUIS LICHTENSTEIN 1930 Micro colorimetric studies II. Estimation of phosphorus: molybdic acid—stannous chloride reagent. J. Biol. Chem., vol. 86, p. 671.

———— 1932 Micro colorimetric studies. III. Estimation of organically bound phosphorus. A system of analysis of phosphorus compounds in blood. J. Biol. Chem., vol. 95, p. 661.

MILROY, T. H. 1931 The present status of the chemistry of skeletal muscular contraction. Physiol. Rev., vol. 11, p. 515.

MORGULIS, SERGIUS AND HOWARD C. SPENCER 1936 a A study of the dietary factors concerned in nutritional muscular dystrophy. J. Nutrition, vol. 11, p. 573.

———— 1936 b A note on the condition of the endocrine glands in myopathy of nutritional origin. Endocrinol., vol. 20, p. 393.

NEVIN, S. 1934 A study of the muscle chemistry in myasthenia gravis, pseudo-hypertrophic muscular dystrophy and myotonia. Brain, vol. 57, p. 239.

OCHOA, SEVERO AND JOSÉ G. VALDECASAS 1929 A micro method for the estimation of total creatinine in muscles. J. Biol. Chem., vol. 81, p. 351.

ROSE, WILLIAM C., OSCAR M. HELMER AND ALFRED CHANUTIN 1927 A modified method for the estimation of total creatinine in small amounts of tissues. J. Biol. Chem., vol. 75, p. 543.

SACKS, JACOB AND WILMA C. SACKS 1933 The fundamental chemical changes in contracting mammalian muscle. Am. J. Physiol., vol. 105, p. 151.

SHAFFER, PHILIP A. AND MICHAEL SOMOGYI 1933 Copper-iodometric reagents for sugar determination. J. Biol. Chem., vol. 100, p. 695.

SOMOGYI, MICHAEL 1930 A method for the preparation of blood filtrates for the determination of sugar. J. Biol. Chem., vol. 86, p. 655.

WENDEL, WILLIAM B. 1933 A note on the determination of lactic and pyruvic acids. J. Biol. Chem., vol. 102, p. 47.

METABOLISM STUDIES IN NUTRITIONAL MUSCULAR DYSTROPHY

S. MORGULIS AND HOWARD C. SPENCER
Department of Biochemistry, University of Nebraska College of Medicine, Omaha

SIX FIGURES

(Received for publication May 16, 1936)

The numerous biochemical investigations of the clinical myopathies indicate that there is a fundamental change in the metabolism of the dystrophic organism. The creatine-creatinine metabolism has received the most attention in these studies, although investigations of the most diverse nature may be found in the literature.

It was considered advisable to study the metabolism of our experimental animals (Morgulis and Spencer, '36) in an effort to determine the fundamental changes which accompany nutritional muscular dystrophy. We studied animals on control diets, on the basic dystrophy producing diet 13 (Goettsch and Pappenheimer, '31), and on the basic diet until the manifestations of dystrophy became evident when this was changed to a recovery diet. Daily records were kept of the body weight of each rabbit; of the weight of food and water consumed; and of the volume, reaction and specific gravity of the urine voided. The urines were analyzed for total nitrogen, creatinine, creatine, phosphorus, chloride and organic acids.

METHODS

The cages used for the metabolic study of rabbits were specially designed, and all the metal parts were chrome-plated. A 10% thymol solution in chloroform was used as a preservative for the urine. The apparatus was cleaned

daily. The urine specimen was acidified with a few drops of H_2SO_4 (which prevented the escape of ammonia and dissolved any precipitated phosphates) and made up to a volume of 150 cc. with the cage washings.

The total nitrogen, creatine and creatinine, and chlorides were determined according to the directions given by Folin ('34). The phosphorus was determined by the Fiske and Subbarow ('25) method and organic acids by Greenwald's ('29–30) modification of the familiar Van Slyke-Palmer procedure.

EXPERIMENTS ON THE CONTROL DIETS

Three different control diets, namely, rabbit-chow, diet 11 and diet 201 were used. Rabbit-chow was chosen since it has been found to be a well-balanced food for rabbits; diet 201 (rabbit-chow treated with ethereal-ferric chloride) was fed to ascertain the effect of ethereal-ferric chloride treatment, per se; while diet 11 (untreated diet 13) was used because of its close similarity to diet 13, the basic dystrophy producing diet.

The results of the experiments on the control diets are presented in figures 1, 2 and 3 and a summary of the metabolism experiments is presented in table 1. Rabbits 306, 311 and 310 maintained on rabbit-chow, diet 11 and diet 201, respectively, appeared perfectly normal throughout the experimental periods. The most rapid gain in weight was made on diet 11, while the slowest gain was obtained on diet 201. Although the water consumption of the three animals varied, in every case the volume of urine voided was very nearly one-half the total water intake. The urine of all three rabbits was alkaline, except for a 2-week span during which the urine of rabbit 310 was neutral to litmus.

The constancy of the elimination of various urinary constituents on these widely different diets was surprising. The most radical difference was found in the case of phosphorus, since none was excreted in the urine on either rabbit-chow or diet 201 (treated rabbit-chow), while an average daily excretion of 38 mg. per kilogram body weight was found in the

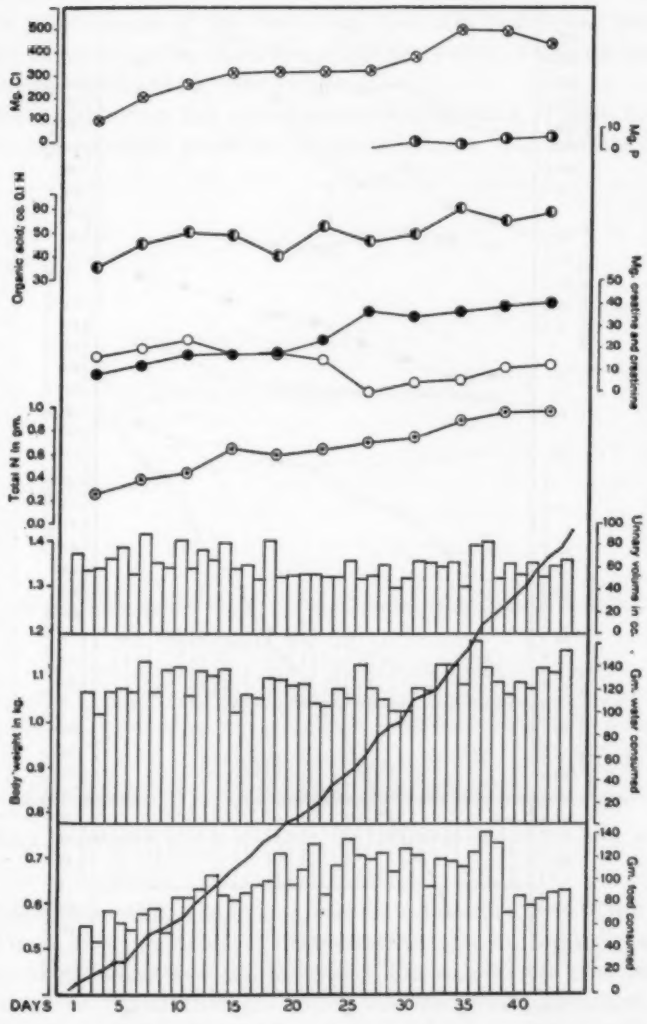

Mg. Cl
Mg. P
Organic acid; cc. 0.1 N
Mg. creatine and creatinine
Total N in gm.
Urinary volume in cc.
Body weight in kg.
Gm. water consumed
Gm. food consumed
DAYS 1 5 10 15 20 25 30 35 40

Fig. 1 Metabolism studies on rabbit 306 maintained on rabbit-chow as a control diet.

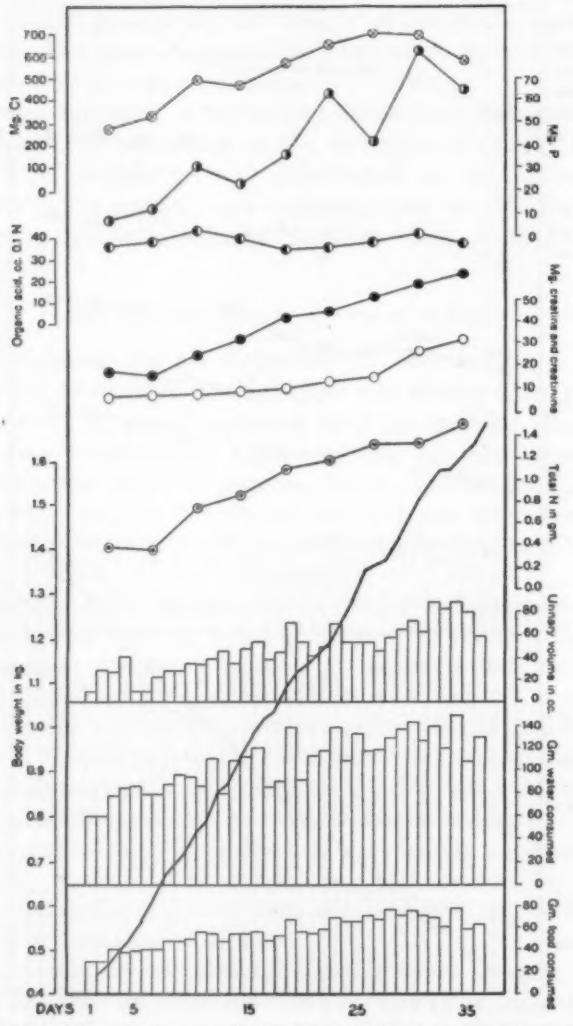


Fig. 2 Metabolism studies on rabbit 311 maintained on diet 11 as a control diet.

animal on diet 11. The chloride excretion was greatest on diet 11 in spite of the fact that diet 201 had been treated with ethereal-ferric chloride, while the lowest chloride excretion was obtained on rabbit-chow.

In considering the nitrogenous constituents, it was found that the greatest positive nitrogen balance was obtained on

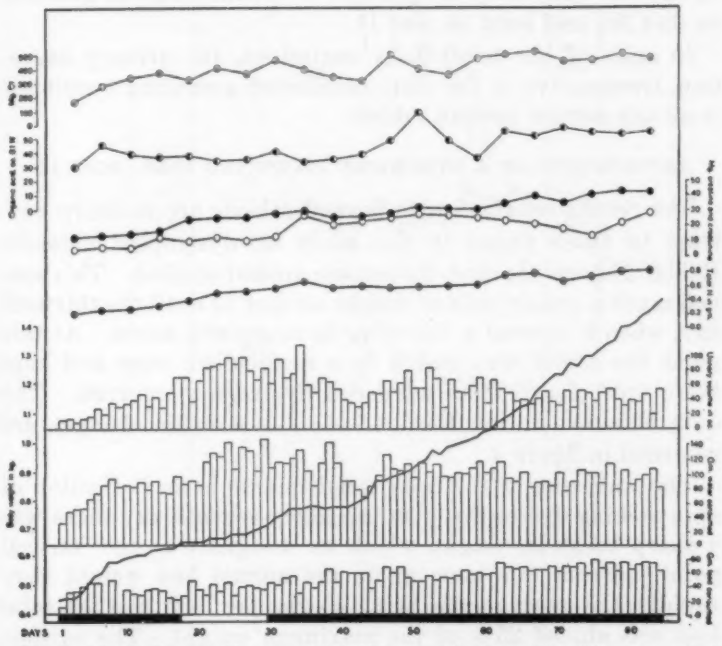


Fig. 3 Metabolism studies on rabbit 310 maintained on diet 201 as a control diet.

rabbit-chow, while the lowest was found on diet 201. This low balance may, possibly, be linked with the exceedingly slow rate of growth given on this diet. Turning to the creatinine, it may be noted that its excretion was quite constant, irrespective of the diet. The average daily excretion of creatinine was 34, 37 and 31 mg. per kilogram body weight on each of the three diets, giving an average for the normal

rabbit of 34 mg. per kilogram. Although the creatine excretion fluctuated more than that of creatinine, nevertheless, its daily excretion in the control rabbits averaged about 17 mg. per kilogram. The creatinine N, calculated as per cent of total N, was also rather constant in the three control rabbits, the highest value being obtained on diet 201 and the lowest on diet 11. Similarly the per cent of creatine N, was greatest on diet 201 and least on diet 11.

In spite of the small daily variations, the urinary excretion, irrespective of the diet, manifested a striking constancy in all our normal control rabbits.

EXPERIMENTS ON A DYSTROPHY PRODUCING DIET (DIET 13)

The results obtained with normal rabbits are in sharp contrast to those found in the study of dystrophic animals. Rabbit 274 was the first dystrophic animal studied. This animal made a steady gain of weight on diet 13 until the thirtieth day, when it showed a wavering in its growth curve. At this point the rabbit was placed in a metabolism cage and kept there until death from advanced dystrophy occurred. The data obtained during this period of dystrophic changes are recorded in figure 4.

The wavering of the body weight curve is an indication of approaching dystrophy. On the thirty-seventh day there was a sharp break in weight, which we designate as the 'critical point.' From this moment on the animal lost weight very rapidly, the average loss being 45 gm. per day, and the total loss was almost 25% of the maximum weight. The earliest outward signs of dystrophy became apparent in this rabbit 3 days after the sharp break in body weight (thirty-ninth day on diet 13) and from thence on developed rapidly until at the time of death the manifestations of dystrophy were manifold.

The sharp break in the weight curve is a very characteristic event in the development of dystrophy. We designate this as the 'critical point' because from this time on the dystrophic process runs a very acute course, soon terminating in death. Although in the matter of details no two rabbits

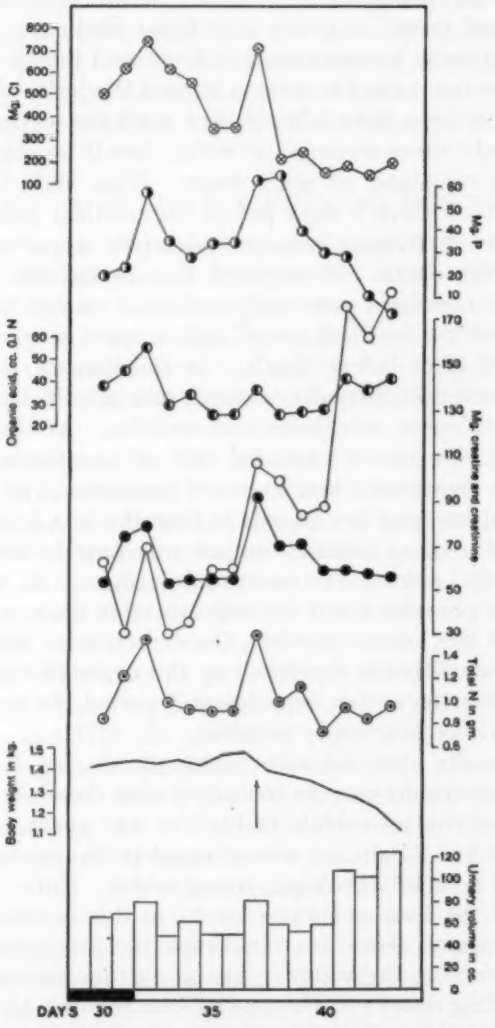


Fig. 4 Metabolism studies on rabbit 274 maintained on the dystrophy producing diet 13.

are alike, all rabbits show the same general behavior, of which the 'critical point' is a very significant landmark. Recovery from dystrophy becomes more difficult and finally impossible the longer the animal is carried beyond the 'critical point.'

The urine was definitely alkaline until the wavering in the body weight curve occurred, at which time it became definitely acid and remained so until death. This shift to an acid reaction took place 5 days before the 'critical point,' 8 days before the dystrophy became outwardly apparent, and 12 days before death. Throughout this period the volume of the urine remained practically constant except for a slight diuresis at the 'critical point' and a more marked diuresis the last 3 days before death. At the time of the 'critical point' there was, however, a sharp rise also in the excretion of total nitrogen, creatinine and creatine. At that time the excretion of creatine exceeded that of creatinine, and this excessive creatinuria became more pronounced as the symptoms of dystrophy developed, so that the last 3 days before death the urinary creatine output was four to five times as great as that prior to the onset of dystrophy. At the 'critical point' the phosphate and chloride excretion likewise increased but, after this initial increase, the excretion of both of these urinary constituents decreased as the dystrophy progressed. Throughout the entire experimental period the organic acid excretion was practically constant.

The results obtained with rabbit 274 during the development of dystrophy may be compared with those obtained with the normal control rabbits (table 1). The average daily loss in weight was 22 gm., or almost equal to the average gain in weight of a rapidly growing, young rabbit. Unfortunately no record of the food and water intake of this animal was kept, and we cannot, therefore, determine the relationship of the urine volume to the water intake nor to the nitrogen intake.

Regarding diet 11 as the control diet for diet 13, the excretion of phosphorus and chloride was diminished in the dystrophic animal. The creatinine excretion was increased by about 30%, while the creatine output increased enormously, at least

200 to 300%. Likewise, the creatinine N, calculated as per cent of total N, increased a little but the creatine N increased two- to threefold. Thus, in the definitely dystrophic animal the striking change is the enormous increase in creatine excretion, accompanied by a lowered excretion of phosphorus and chloride.

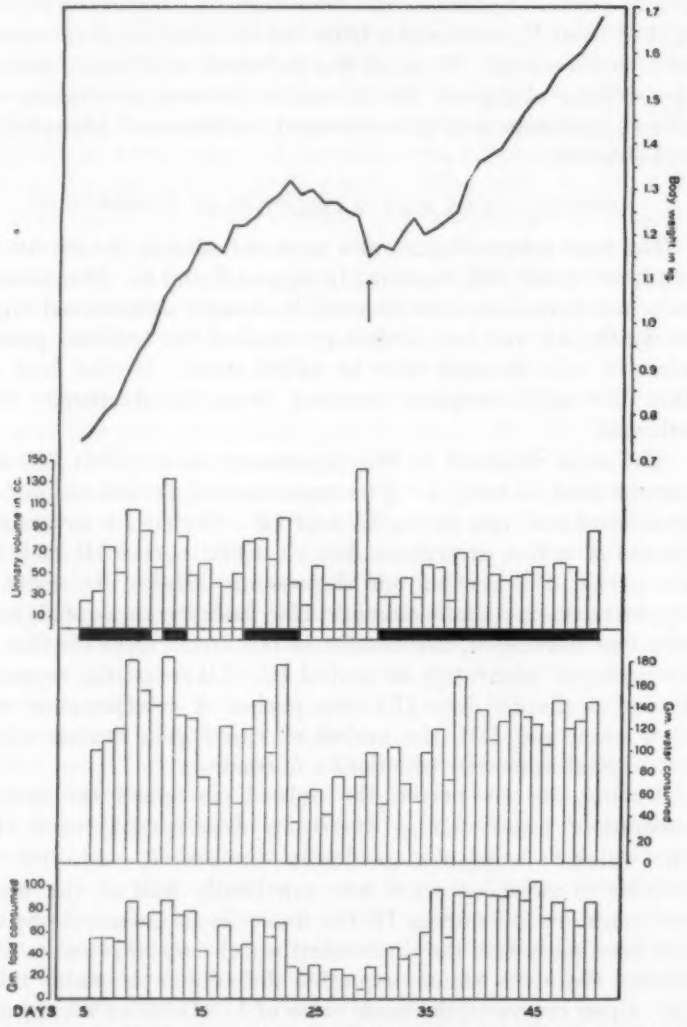
EXPERIMENTS ON DIET 13 FOLLOWED BY RABBIT-CHOW

The most interesting results were obtained in the metabolic study of rabbit 303, recorded in figures 5 and 6. This animal was maintained on diet 13 until it showed pronounced signs of dystrophy and had definitely reached the 'critical point,' when it was changed over to rabbit-chow. It was kept on this diet until complete recovery from the dystrophy was attained.

The data obtained in the experiment with rabbit 303 are summarized in table 1. The experimental period on diet 13 is divided into two parts, IA and IB. Period IA covers the period of active growth on diet 13, while period IB includes the period of wavering and decreasing weight. In order to appraise the metabolic changes after definite signs of dystrophy had developed, the results of the last 3 days on diet 13 are grouped separately as period IC. Likewise, the recovery period is divided into IIA, the period of readjustment and slow gain, and IIB, the period of rapid gain during which the animal appeared practically normal.

During the IA period the animal consumed the largest amount of water, but as dystrophy developed (period IB), the water consumption gradually diminished. At first the volume of urine excreted was practically half of the water consumption but during IB the decrease in urine volume did not keep pace with the diminished water consumption, so that finally the urine volume equalled the volume of water taken in. Upon recovery, the usual ratio of 1:2 between the urinary output and water intake was rapidly restored.

As the animal became dystrophic its food consumption gradually decreased. However, its nitrogen excretion did not



Figs. 5 and 6 Metabolism studies on rabbit 303 in which dystrophy was produced by means of diet 13 and then recovery effected by the feeding of rabbit-chow.

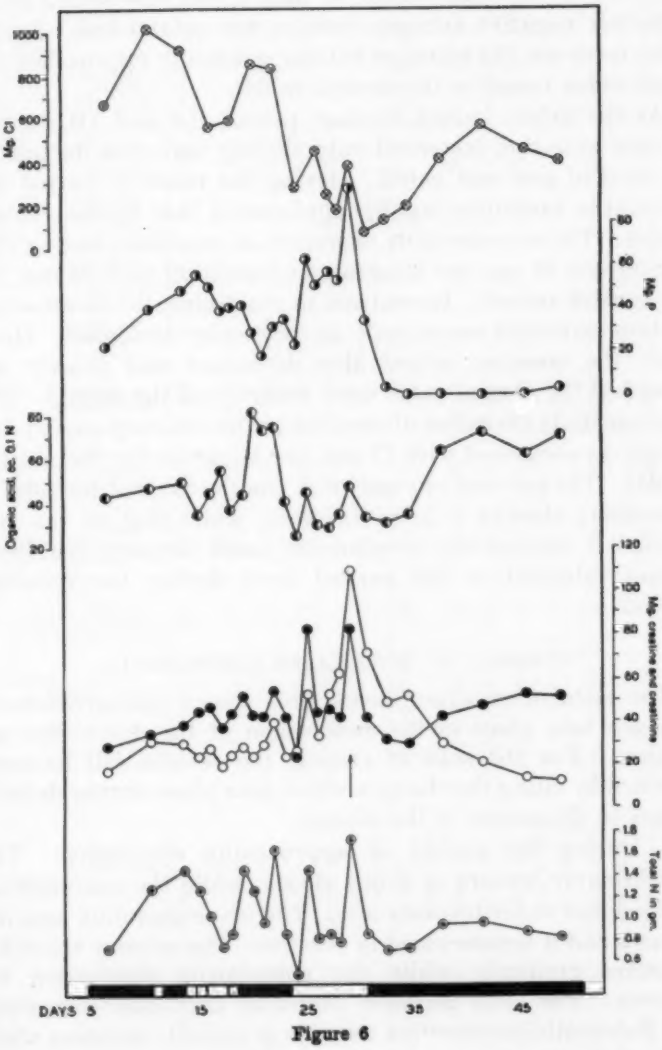

Mg Cl
Mg P
Organic acid, as 0.1 N
Mg creatine and creatinine
Total N in gm
DAYS 5
15
25
35
45

Figure 6

decrease to the same extent, so that during the period IC, a definitely negative nitrogen balance was established. Again, upon recovery, the nitrogen balance gradually approached the same value found in the control rabbit.

As the rabbit passed through periods IA and IB its creatinine excretion increased only slightly and even during IC no marked rise was noted. During the recovery period the creatinine excretion rapidly approached that in the control rabbit. The average daily excretion of creatinine during this period was 30 mg. per kilogram as compared with 34 mg. for the control animal. In contrast to creatinine, the creatine excretion increased enormously as dystrophy developed. However, the creatine output also decreased and quickly approached the normal value upon recovery of the animal. The average daily excretion of creatine in the recovery period was 19 mg. as compared with 17 mg. per kilogram for the normal rabbit. The per cent of creatine N (on the basis of the total N excretion) showed a great increase, while that of the creatinine N showed only a relatively small increase, but these values returned to the normal level during the recovery period.

SUMMARY OF METABOLISM EXPERIMENTS

The metabolism experiments have shown that pronounced changes take place in the metabolism of the dystrophic organism. For the sake of clarity, the results will be summarized by citing the changes which take place during definite stages in the course of the disease.

1. During the period of approaching dystrophy: The weight curve wavers or drops slowly, while the consumption of food and water becomes less. The urine excretion also decreases and it becomes acid in reaction. The urinary chlorides decrease gradually while the phosphorus elimination increases. The total nitrogen excretion decreases somewhat, but the creatinine excretion remains practically constant while that of creatine increases by leaps and bounds.

2. At the 'critical point': The body weight declines sharply. The ratio of urine output to the water intake approaches the

TABLE 1

Summary of metabolism experiments

RABBIT	DIET	DAYS ON DIET	REMARKS	AVERAGE WEIGHT IN GRAMS	AVERAGE DAILY GAIN OR LOSS IN WEIGHT IN GRAMS	FOOD CONSUMPTION GRAMS/DAY/KILOGRAMS	WATER CONSUMPTION GRAMS/DAY/KILOGRAMS	URINE OUTPUT CUBIC CENTIMETERS/DAY/KILOGRAMS	DAILY URINARY EXCRETION							
									Total N grams/kilograms	Creatinine milligrams/kilograms	Creatine milligrams/kilograms	Total P milligrams/kilograms	Total Cl milligrams/kilograms	N Balance grams/kilograms	Creatinine-N per cent of total N	Creatine-N per cent of total N
306	Rabbit-chow	44	Normal	850	+ 22.0	113	141	70	0.762	34	19	0	382	+ 1.7	1.5	0.8
311	11	36	Normal	1080	+ 34.4	52	102	46	0.930	37	13	38	537	+ 1.1	1.3	0.5
310	201	84	Normal	900	+ 12.5	55	110	55	0.620	31	20	0	456	+ 0.6	1.7	1.1
Average				943	+ 23.0	73	118	57	0.771	34	17		458	+ 1.1	1.5	0.8
274	13	14	Period of definite dystrophy	1300	— 22.0			56	0.800	49	68	25	302		2.0	2.9
303	13	25	Period I dystrophy	1130	+ 15.6	43	89	55	0.970	37	29	36	571	+ 0.7	1.3	1.0
303	13	10	Period IA	964	+ 34.0	72	141	72	1.030	34	27	36	908	+ 1.8	1.1	0.9
303	13	15	Period IB	1245	— 2.0	31	67	47	0.865	39	30	37	410	+ 0.4	1.5	1.2
303	13	3	Period IC (last 3 days of period IB)	1213	— 33.0	14	56	56	0.895	45	107	55	220	— 0.3	1.9	3.3
303	Rabbit-chow	21	Period II recovery	1450	+ 25.2	50	70	35	0.610	30	19	1	265	+ 0.5	1.7	1.0
303	Rabbit-chow	8	Period IIA	1210	+ 12.5	40	72	36	0.680	30	36	5	203	+ 0.2	1.5	1.8
303	Rabbit-chow	13	Period IIB	1575	+ 33.0	58	87	35	0.600	32	11	Trace	309	+ 0.7	1.7	0.6

value of 1:1 as the acidotic metabolism develops. There is marked diuresis accompanied by a relatively slight increase in creatinine, total nitrogen, phosphorus and chloride excretion and an enormous increase in the creatine elimination. At this point the creatine N, calculated as per cent of total N, doubles or trebles itself and the nitrogen balance becomes definitely negative.

3. During the period of progressive dystrophy, i.e., from the 'critical point' to the time of death: The weight continues to fall rapidly. The phosphorus and chloride excretion gradually decrease. The creatinine and total nitrogen decrease from the high level reached at the 'critical point,' while the creatine excretion continues to rise till the time of death. The urine volume decreases following the brief strong diuresis at the critical point, but just before death a marked diuresis occurs again.

4. During the period of recovery, following a change of diet at the 'critical point': The weight increases at first slowly, then very rapidly. The food and water intake increases gradually. The ratio of the urine output to water intake soon returns to the normal value of 1:2. The urine almost immediately becomes alkaline. The excretion of phosphorus and chloride approaches the values normal for that diet. The nitrogen balance becomes positive and the creatine elimination approaches the normal level. Meanwhile, the rabbit gradually loses the symptoms of dystrophy and behaves perfectly normal.

LITERATURE CITED

FISKE, CYRUS H. AND YELLAPRAGADA SUBBAROW 1925 The colorimetric determination of phosphorus. J. Biol. Chem., vol. 66, p. 375.

FOLIN, O. 1934 Laboratory manual of biological chemistry. D. Appleton-Century Co., New York.

GOETTSCH, MARIANNE AND ALWIN M. PAPPENHEIMER 1931 Nutritional muscular dystrophy in the guinea pig and rabbit. J. Exp. Med., vol. 54, p. 145.

GREENWALD, ISIDOR 1929–1930 Studies on metabolism in pneumonia. I. The excretion of 'organic acid' and a method for its determination. J. Biol. Chem., vol. 85, p. 447.

MORGULIS, SERGIUS AND HOWARD C. SPENCER 1936 A study of the dietary factors concerned in nutritional muscular dystrophy. J. Nutrition, vol. 11, p. 573.

THE EFFECT OF SELENIUM CONTAINING FOODSTUFFS ON GROWTH AND REPRODUCTION OF RATS AT VARIOUS AGES[1]

KURT W. FRANKE AND VAN R. POTTER[2]
Department of Experiment Station Chemistry, South Dakota State College, Brookings

THREE FIGURES

(Received for publication April 2, 1936)

In the assay of grain samples (Franke, '34) it was observed that rats which were several days older than the other rats in the group usually survived much longer on the toxic diet. In this report it was stated that 71.1% of the rats placed on lethal samples of grain were dead by the sixtieth day of the experiment. The rats were placed on experiment at ages varying from 27 to 31 days.

In a preliminary trial (series 69) to test out the theory that older rats were much more resistant to the toxicant, three adult male rats were placed in a cage and given the standard diet made with no. 582 wheat (lethal). The animals were sacrificed on the 60th, 120th and 360th days of the experiment. No outstanding gross pathology was present in these rats.

Another group (series 76) of six males was selected and placed on a control wheat diet. They were then shifted to the no. 582 wheat diet at the ages of 60, 70, 80, 90 and 100

[1] Published with the permission of the director of the South Dakota Agricultural Experiment Station as communication no. 22 from the Department of Experiment Station Chemistry and is part 14 of "A New Toxicant Occurring Naturally in Certain Samples of Plant Foodstuffs." These investigations are being carried out under the Purnell Fund.

[2] Wisconsin Alumni Research Foundation Fellow, Madison, Wisconsin.

days, respectively. All of the rats showed appreciable weight losses for the first few days after the change in diet. Growth was then resumed, but at a somewhat depressed rate. The animals were sacrificed after 360 days on the diet. None of the rats had died. Post mortems revealed varying degrees of atrophy and regeneration in the liver. None of the rats were anemic.

A group of very young rats was placed on the toxic diet (no. 582 wheat) at the age of 20 to 22 days (series 90). Death occurred with acute symptoms in four out of five cases before the twenty-second day of experiment. The remaining rat died on the fifty-second day with distinct atrophy of the liver.

These preliminary trials showed that a marked increase in resistance occurred between the twentieth and the sixtieth day of life in the case of the rat. A long-time experiment (series 99) was accordingly planned to show the effects of the toxicant on rats from weaning age to maturity.

EXPERIMENTAL

Forty-two rats were weaned when 21 days of age and placed in seven groups of six rats each (two males and four females). The rats were also divided as evenly as possible according to litter and weight, and placed in individual drawer cages.

Group I was shifted at once to the toxic no. 582 wheat diet (24.6 ppm. of naturally occurring Se). The remaining six groups were placed on the control wheat diet. Groups II, III, IV, V and VI were shifted to the toxic diet when the rats were 42, 63, 84, 105 and 186 days of age, respectively. Group VII remained on the control diet throughout the experiment. All of the rats served as controls until they were shifted to the toxic diet.

After each group had been on the toxic diet for a period of 40 days, the rats were placed in large breeding cages for a period of 14 days after which they were replaced in their individual cages.

During the breeding period the males on the toxic diet were placed with stock females from 1 to 8 P.M. daily, the stock diet being removed. It was thus possible to mate the males with both affected and normal females. Group VI was used as a control for the matings during the period from 82 to 131 days of age, while group VII was used during the period of 145 to 226 days of age. By this time group VI had served its purpose in controlling the earlier experimental matings and was now shifted to the toxic diet, and 40 days later the rats were mated, using group VII for controls.

In order to make a cross breeding of control males with affected females another series of four groups of four females (series 117) was fed and handled to correspond to groups II, III, IV and V of series 99.

The rats in both series were weighed at 5-day intervals, and food intake was recorded daily. Fresh distilled water containing a trace of iodine was available at all times. The composition of the diet has been given in a former publication (Franke and Potter, '34). Careful autopsies were made of all rats at death.

DISCUSSION OF RESULTS

The growth of the animals is shown in figures 1 and 2. The rats in group I which were placed on the toxic diet at the age of 21 days were completely unable to withstand the toxic effects of the diet. All were dead by the twenty-first day of experiment. The average survival was 13 days. All of the animals showed acute symptoms (Franke, '34).

In striking contrast to the animals in group I, the animals in group II, which were placed on the toxic diet at the age of 42 days, not only failed to die in the time which caused death for group I, but were still alive after 40 days. Franke ('34) showed that in the case of 325 rats which were placed on lethal grain samples at the age of 28 days, 38.5% were dead by the fortieth day. Thus it is apparent that a marked increase in resistance to the toxicant occurred between the twenty-first and forty-second day of life, and much of this

between the twenty-eighth and forty-second day. Group II was nevertheless greatly affected by the toxicant and responded by an immediate restriction of food intake (fig. 3) and a sharp loss in weight (figs. 1 and 2). After the preliminary weight loss an equilibrium was apparently reached,

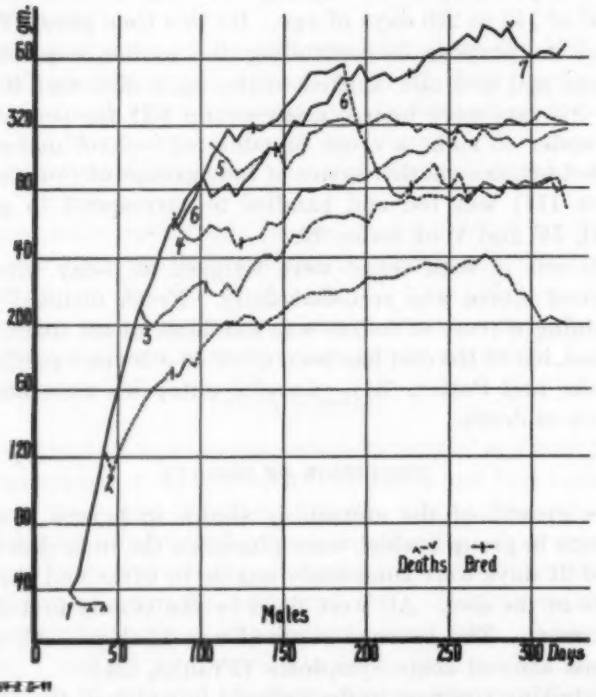


Fig. 1 Growth of male rats. Showing effect of toxic diet on rats of various ages, as follows: Group 1, 21 days; 2, 42 days; 3, 63 days; 4, 84 days; 5, 105 days; and 6, 186 days old. Average weight of two.

and the rats continued at a subnormal and irregular rate of growth, with all dead by the 225th day. The behavior of the remaining groups was strictly analogous to that of group II, except for longevity of life.

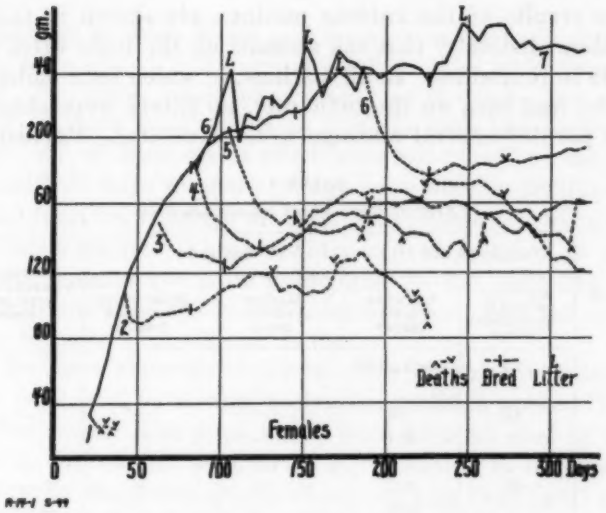


Fig. 2 Growth of female rats. Showing effect of toxic diet on rats of various ages, as follows: Group 1, 21 days; 2, 42 days; 3, 63 days; 4, 84 days; 5, 105 days; and 6, 186 days old. Average weight of four.

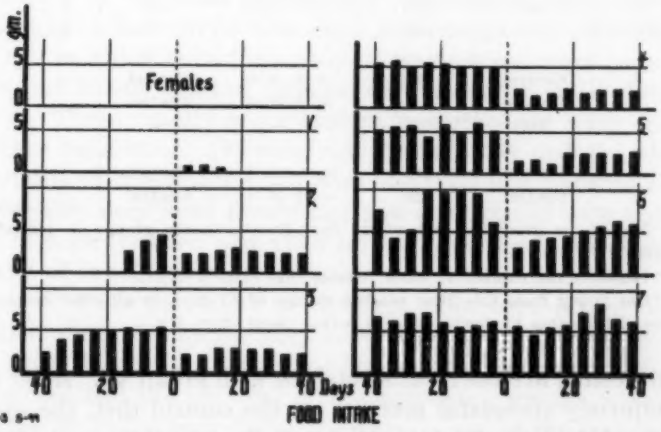


Fig. 3 Average food intake for 40 days before and after shifting from control to toxic diets, with the following exceptions: Group 1 was not on the control diet at all, group 2 was on the control diet less than 40 days, and group 7 was on the control diet throughout.

The results of the various matings are shown in table 1, and show distinctly that the animals on the toxic diets were unable to reproduce. In all matings, in which both males and females had been on the toxic diet, no litters were obtained, while parallel control matings were successful. Particularly

TABLE 1

Effect of toxic wheat on reproduction

GROUP NO.	SERIES 99 AND 117		SERIES 99		SERIES 117, FEMALES MATED WITH STOCK MALES
	Age when shifted to toxic diet (days)	Age when bred (days)	Matings within group	Males mated with stock females	
1	21	Dead at 42			
2	42	82	— — — —[1]	—[1]	— — — —
		131	— — — —	—	
3	63	103	— — — —	+	+ — — —
		250			— — — —
4	84	124	— — — —	+	+ + + +
		320			+ — — —
5	105	145	— — — —	+	+ — — —[2]
		250			+ — — —
6	Control	82	+ + + +	Control	
	Control	131	+ + + +	Control	
	186	226	— — — —	+	
7	Control	145	— + + +	Control	
	Control	226	— + + +	Control	

[1] Results are given for each of the four females in the group; + indicates a litter.

[2] Indicates the number of stock females that caste a litter.

[3] One young from this litter reached an age of 61 days, in all other cases pups were either eaten by female or died before twenty-first day.

interesting are the results obtained with group VI. After two uniformly successful matings on the control diet, the group was shifted to the toxic diet. Following the usual 40-day period, the animals were mated again, but the matings were uniformly unsuccessful, no litters being cast. In addition to

the matings between males and females on the toxic diet, the males were mated with normal stock females of proven fertility. The males which were placed on the toxic diet at the age of 42 days were unable to fertilize even the normal females. All of those males which were not placed on the toxic diet until 63 days or more of age were able to fertilize the normal females, although matings with females on toxic diets were unsuccessful. These findings cannot be taken to mean that the females are more susceptible to the toxicants than the males since the affected females were not given the opportunity to mate with normal males.

In the later experiment (series 117) in which affected females were bred by normal males, the matings proved fertile in a few cases. The pups which were born all died or were eaten by the mother soon after birth, except in the case of one litter in the fourth group where one pup lived to 61 days. The growth of the rats in series 117 was almost exactly parallel with that of the animals in series 99.

These results show that animals which receive toxic diets are unable to reproduce successfully. Matings in which both animals have been given toxic diets were completely infertile. Matings in which one of the animals was normal were sometimes fertile, but affected females were unable to raise their young. These results are therefore in agreement with our previous suggestions (Franke and Potter, '35) and the observations of Macomber ('23) who observed that animals of low fertility may yield fertile matings were placed with animals of high fertility, and yield infertile matings with other animals of low fertility.

The older the rats were at the time they were placed on the toxic diets, the longer they survived. The following figures show the marked difference between the survival of the first group and the survival of the remaining groups which were placed on the toxic diet. The average age of the females in the various groups in series 99 are given: I, 32 days; II, 211 days; III, 279 days; IV, 247 days; V, 383 days; VI, 512 days; and VII, 601 days. These averages include the

following survivors which were killed on the 646th day of experimentation: group III, 1; IV, 2; VI, 1 and VII, 4.

Steyn ('34) has discussed the relation between the action of poisons and the age of animals and states that, with the exception of animals in extreme age, the younger the animal the more susceptible it is to the action of poisons. However, MacKay and MacKay ('30) believe that food intake may be a determining factor. Contrary to the results of Cox, Smythe and Fishback ('29) they came to the conclusion that cystine added to the diet is no more toxic for young than for older rats. They considered the apparent difference in effect to be due to the fact that the younger rats ingested more food, and hence more cystine, in relation to their body weight than did older rats. In our own experiments the remarks of MacKay and MacKay would not apply, since the restriction of food intake, which always occurs when toxic wheat is fed, was particularly pronounced in the case of the youngest animals which were placed on the diet. Although inanition undoubtedly hastened the death of the animals in this group, there can be no question but that the younger rats are more susceptible to the toxicant than the older rats.

In checking over the post mortem records from series 99 it was found that very little lung infection occurred before the 300th day of the experiment. Of eighteen rats which died in this period, only two showed definite lung infection, and one of these was a control animal. There was some pleural edema in seven cases. Of the twenty-four rats which died after the 300th day, seven rats, including one control, showed lung infection. Although this data is far from conclusive, it suggests the possibility that the toxicant may render the animals less resistant to infection. The fact that the affected animals are in an extremely poor nutritive condition due to the effects of the toxicant undoubtedly lowers their resistance.

At death the rats in group I showed the typical acute symptoms which have been previously described (Franke, '34). The remainder of the affected rats died with the usual

chronic symptoms. The livers showed atrophy of the central, caudate and left lateral lobes, while the right lateral was hypertrophied in many cases.

The failure of the affected rats to reproduce made it seem quite likely that marked atrophy of the reproductive organs might be found. It is very difficult to make comparisons between the organ weights of normal and affected rats. Even when the weights of the organs are recalculated to 100 gm. of body weight the fact that the controls have body fat while the affected rats are emaciated causes a disparity in the figures. Cases of atrophy are quite likely to be valid, however, since the above effect would be to diminish rather than to increase the differences between the weights of normal and affected organs. A study of the records for the males failed to reveal any marked deviations from the normal. In the case of the females, however, the reproductive organs were definitely under-developed in a number of cases. It appeared that very little development of the female reproductive organs occurred after the females had been placed on the toxic diet. The mating records indicated reproductive failure in cases which were not revealed by the post mortem examinations.

The results of this paper would seem to be of considerable economic importance. Animals which are approaching maturity may have their reproductive powers injured by the feeding of toxic grains, while their weight losses may not be large enough to arouse attention. Furthermore, older animals thrive better on a toxic foodstuff and may show weight gains which cannot be described as subnormal when a control is not present for comparison. Yet the subnormal gains may destroy the margin of profit for the livestock feeder.

SUMMARY

1. White rats were placed on toxic wheat diets at ages varying from 21 to 186 days, and studied from the standpoint of growth, reproduction, and mortality.

2. There is a rapid increase in resistance to the toxicant between the ages of 21 and 42 days. Animals placed on toxic diets at 21 days lost weight and died in less than 20 days, while rats which were placed on toxic diets at 42 days of age or older lived for more than 200 days in most cases.

3. Rats which were able to survive on the toxic diets for relatively long periods of time showed subnormal growth and a distinct loss in reproductive powers. Matings in which both animals were fed toxic wheat were completely infertile. Matings in which one animal was normal were sometimes fertile, but affected females were unable to raise their young.

4. The economic significance of the results was discussed.

LITERATURE CITED

COX, GERALD J., C. V. SMYTHE AND C. F. FISHBACK 1929 The nephropathogenic action of cystine. J. Biol. Chem., vol. 82, pp. 95–103.

FRANKE, KURT W. 1934 A new toxicant occurring naturally in certain samples of plant foodstuffs. I. Results obtained in preliminary feeding trials. J. Nutrition, vol. 8, pp. 597–608.

FRANKE, KURT W. AND V. R. POTTER 1934 Ibid. III. Hemoglobin levels observed in white rats which were fed toxic wheat. J. Nutrition, vol. 8, pp. 615–624.

———— 1935 Ibid. IX. Toxic effects of orally ingested selenium. J. Nutrition, vol. 10, pp. 213–221.

MACKAY, EATON M. AND LOUIS LOCHARD MACKAY 1930 Age and the effect of unusual diets. J. Biol. Chem., vol. 86, pp. 765–771.

MACOMBER, DONALD 1923 The threshold of fertility in rats and its relation to dietary deficiency. Am. Naturalist, vol. 57, p. 519.

STEYN, DOUW G. 1934 The toxicology of plants in South Africa. Onderstepoort J. Vet. Sci. and Animal Ind., vol. 3, pp. 359–473.

THE EFFECT OF THE ADMINISTRATION OF SODIUM BICARBONATE AND OF AMMONIUM CHLORIDE ON THE AMOUNT OF ASCORBIC ACID FOUND IN THE URINE

ESTELLE E. HAWLEY,[1] JOHN P. FRAZER, LUCIUS L. BUTTON AND D. J. STEPHENS

Departments of Pediatrics and Medicine, University of Rochester School of Medicine and Dentistry, Rochester

TWO FIGURES

(Received for publication May 16, 1936)

In a previous study of the excretion of vitamin C in the urine of normal subjects, individual variations were observed in the response to repeated test doses of ascorbic acid, both during and after apparent saturation with the vitamin (Hawley, Stephens and Anderson, '36). It was suggested that factors not satisfactorily controlled might be responsible for the observed variations. A chance observation in a patient with hemorrhagic nephritis led to the suspicion that the variations in the hydrogen ion concentration of the urine might be accompanied by variations in the urinary excretion of vitamin C. The experiment recorded in this communication was designed to investigate the validity of this hypothesis.

METHOD

Two normal young men were chosen as subjects. Throughout the duration of the experiments they remained in good health and continued their usual activities as medical students. Except for a short period at the conclusion of the experiment,

[1] Aided by a grant from the California Fruit Growers Exchange

A preliminary report of this investigation appeared in Proc. Exp. Biol. and Med., 1936, vol. 34, p. 218.

a diet practically devoid of vitamin C was taken throughout the period of observation. The daily diet consisted of the following: 50 gm. of crackers, 400 gm. of bread, 27 gm. of cereal, 100 gm. of apple butter, 50 gm. of milk chocolate, 75 gm. of cheese, 50 gm. of butter, 25 gm. of sugar, 1 liter of milk, 126 cc. of 4-X cream, 200 cc. of consomme soup, 90 gm. of beefsteak. To this basal diet measured quantities of vitamin C were added as desired in the form of orange juice, ascorbic acid by mouth or ascorbic acid intravenously,[2] given immediately after breakfast.

Variations in the hydrogen ion concentration of the urine were induced by the ingestion of measured amounts of sodium bicarbonate or ammonium chloride. Twenty-five grams of sodium bicarbonate and 4 gm. of ammonium chloride, taken in divided doses during the day, resulted in the excretion of a markedly alkaline and a markedly acid urine, respectively. No significant changes in the carbon dioxide combining power of the blood resulted from the administration of sodium bicarbonate or ammonium chloride in these amounts. When a vitamin C preparation was given by mouth a period of at least 2 hours was allowed to elapse between the administration of the acid or alkaline salt and the vitamin C preparation.

The urine was collected in 24-hour periods, from 8 A.M. to 8 A.M. Each specimen as voided was immediately divided into two exactly equal portions. One portion, for the estimation of vitamin C, was deposited in a dark brown bottle containing 15 cc. of glacial acetic acid; the bottle was then stoppered and stored in the ice box. We have shown that when urine is stored under such conditions there is less than 5% loss of vitamin C in 24 hours. The second portion of each voided sample was stored in the ice box in a second collection bottle without preservative. At the end of the 24-hour period the hydrogen ion concentration of the pooled sample stored without preservative was determined by the colorimetric method. A number of observations showed that there was no

[2] We are indebted to Merck and Company for providing us with the 'Cebione' (ascorbic acid) for this experiment.

significant change in the pH of the urine when kept for a period of 24 hours under such conditions. The vitamin C content of the acidified sample was determined by rapid titration of the urine against a measured volume of a standardized solution of 2:6 dichlorophenolindophenol according to the method described by Birch, Harris and Ray ('33) and expressed as milligrams per 24 hours.

RESULTS

The variations in the urinary excretion of ascorbic acid and in the hydrogen ion concentration of the urine resulting from variations in the intake of vitamin C, sodium bicarbonate and ammonium chloride are shown in figures 1 and 2.

At the beginning of the experiment the 24-hour excretion of ascorbic acid was determined during a single control day on the basal diet. This was followed by the daily administration of 400 mg. of ascorbic acid in the form of 800 cc. of orange juice for a period of 7 days. At the end of this time each subject was excreting approximately 75% of the daily test dose in the urine, indicating that saturation with the vitamin had occurred. Reduction in the daily dose of acorbic acid to 200 mg. resulted in a comparable reduction in the amount of the vitamin appearing the urine. The addition of sodium bicarbonate was followed by an increase in the pH of the urine from the control level of approximately 6.5 to approximately 7.0; this was accompanied by a decrease in the urinary excretion of the vitamin to approximately 50% of the test dose. The period between November 30th and December 10th, during which the daily ration of vitamin C was provided as ascorbic acid, is particularly significant. During the period of sodium bicarbonate administration the urine was highly alkaline and contained approximately 50% of the daily test dose. The substitution of ammonium chloride for sodium bicarbonate resulted in the excretion of a highly acid urine and a marked increase in the daily excretion to approximately 100% of the daily dose of vitamin C.

The remainder of the experiment was devoted to observations of the effect of repeated withdrawal and readministration of vitamin C during periods of alkali and acid administration. Under comparable conditions of vitamin intake, the

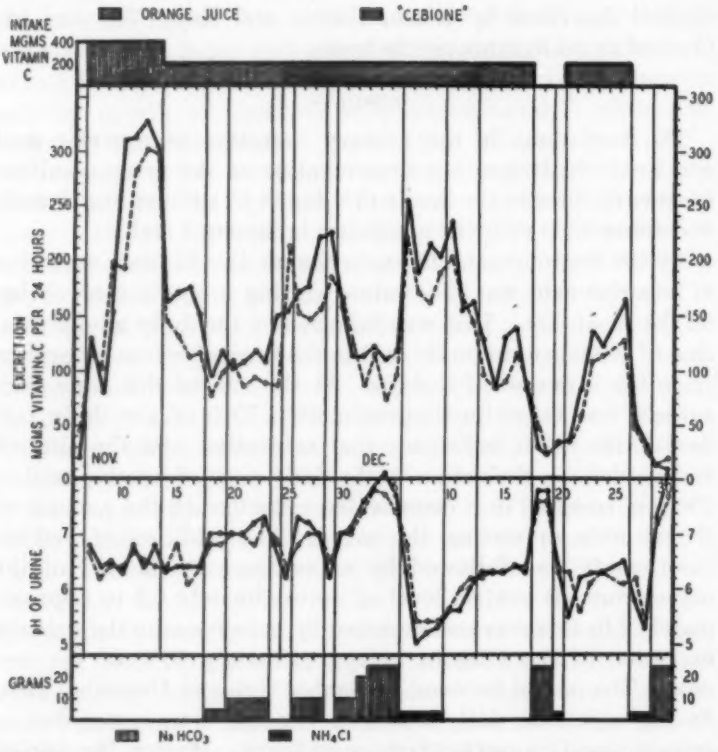


Fig. 1 The effect of the administration of orange juice, crystalline ascorbic acid, sodium bicarbonate and ammonium chloride on the amount of ascorbic acid found in the urine and on the hydrogen ion concentration of the urine. The solid line represents observations in the case of subject L.B. The broken line represents observations made in the case of subject J.F.

urinary excretion of ascorbic acid was consistently greater during periods of ammonium chloride ingestion with a highly acid urine as compare with those periods when sodium bicarbonate was being taken and the urine was highly alkaline.

This held true during periods of depletion as well as during periods of saturation with the vitamin.

At the conclusion of the experiment, both subjects took an uncontrolled diet containing adequate amounts of antiscorbutic foods for a period of 12 days; at the end of this time the excretion of approximately 60 and 100%, respectively, of a test dose of ascorbic acid indicated that a state of saturation

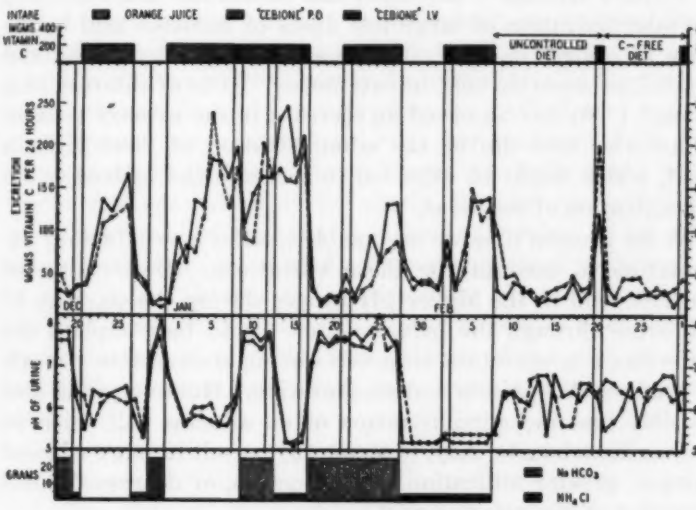


Fig. 2 Legend as in figure 1.

had been maintained by such a diet. During a subsequent 9-day period of vitamin C-free diet the daily 24-hour excretion remained approximately the same as when relatively small but adequate amounts of antiscorbutic foods were being taken. At the end of this period, however, no increase in the control level was observed after a test dose of 200 mg. of ascorbic acid, suggesting that tissue reserves had been depleted.

DISCUSSION

It may be concluded from the above observations that the urinary excretion of ascorbic acid is increased by the administration of ammonium chloride in amounts sufficient to render the urine highly acid and is decreased by the administration of sodium bicarbonate in amounts which result in a highly alkaline urine. The effect was observed during periods of relative vitamin C depletion and saturation and following the administration of large test doses of ascorbic acid in the form of orange juice, crystalline ascorbic acid by mouth and crystalline ascorbic acid intravenously. It is of interest that Ahmad ('36) has observed an increase in the urinary content of ascorbic acid during the administration of diets high in meat, which might be expected to increase the hydrogen ion concentration of the urine.

At the present time we are unable to offer a satisfactory explanation to account for these variations. Destruction of ascorbic acid in the higher pH ranges during the passage of the urine through the urinary tract would best explain the differences in excretion observed during comparable periods of vitamin C depletion and resaturation. However, it is also possible that the administration of an alkaline salt, such as sodium bicarbonate, may, in some way, result in more efficient storage, greater utilization or destruction, or decreased renal excretion of the ascorbic acid.

Euler, Myrbach and Larsson ('33) have shown that the rate of oxidation of ascorbic acid is greatly increased in the higher pH ranges. In view of the fact that the amounts of sodium bicarbonate and ammonium chloride administered were not sufficient to induce significant changes in the carbon dioxide combining power of the blood, it is considerably unlikely that the variations observed were due to any differences in destruction of the vitamin within the body. The changes in the hydrogen ion concentration of the urine, however, were prompt and striking and closely correlated with the variations in ascorbic acid excretion. It is hoped that a series of animal and in vitro experiments now in progress may help to clarify the

mechanism responsible for the observed effect of the administration of acid and alkali on the amount of ascorbic acid found in the urine.

The practical implications of these observations are limited by the fact that the pH of normal urine, as voided, varies between 4.8 and 7.4, with an average of about 6. The rate of oxidation of ascorbic acid at such hydrogen ion concentrations is relatively slow and the differences in the observed ascorbic acid content of the urine as voided might be expected to be negligible. Under conditions which result in the excretion of a highly alkaline urine, however, the diminution in the amount of ascorbic acid found, determined by the method used in these experiments, may amount to 50%, as compared with that excreted under comparable conditions in a highly acid urine. These findings demonstrate clearly that in evaluating the results obtained by studying the vitamin C content of the urine in any individual, one should know not only the dietary history of that individual but the approximate pH of the urine at the time of study. Discrepancies in findings may be dependent upon changes in the acid base balance of the diet or upon metabolic disturbances which may have a similar urinary effect.

SUMMARY

1. The effect of the administration of sodium bicarbonate and ammonium chloride, in amounts sufficient to induce marked changes in the hydrogen ion concentration of the urine, on the excretion of ascorbic acid has been studied in two normal young adults under controlled conditions of vitamin C depletion and saturation.

2. The ascorbic acid content of the urine was consistently and significantly lower during periods of sodium bicarbonate administration, when the urine was highly alkaline, as compared with similar periods of ammonium chloride administration with a highly acid urine.

LITERATURE CITED

AHMAD, B. 1936 Observations on the excretion of vitamin C in human urine. Biochem. J., vol. 30, p. 11.

BIRCH, T. W., L. J. HARRIS AND S. N. RAY 1933 A microchemical method for determining the hexuronic acid (vitamin C) content of foodstuffs, etc. Biochem. J., vol. 27, p. 590.

EULER, H., K. MYRBACH AND H. LARSSON 1933 Sauerstoffaufnahme durch Vitamin C-haltige Organe und durch Gluco-Reduktone. Zeitschr. f. physiol. Chem., Bd. 217, S. 1.

HAWLEY, E. E., D. J. STEPHENS AND G. ANDERSON 1936 The excretion of vitamin C in normal individuals following a comparable quantitative administration in the form of orange juice, cevitamic acid by mouth and cevitamic acid intravenously. J. Nutrition, vol. 11, p. 135.